The effects of nutrient supply on the pattern of food intake in sheep

Thesis

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THE EFFECTS OF NUTRIENT SUPPLY ON THE PATTERN OF FOOD INTAKE IN SHEEP

BY

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ABSTRACT

Voluntary food intake (VFI) in ruminants has been extensively researched. Many factors are involved and inter-related, making theories complex. The rumen affects feedback signals controlling food intake, both short-term and long-term. Control of grass silage intake is associated with specific factors, including ensiling end-products, and asynchrony of ruminal nutrient release. Synchronising energy and nitrogen (N) supply to rumen micro-organisms can improve rumen degradability efficiency, which may alter VFI. However, few studies have investigated effects of ruminal nutrient release on intake patterns of grass silage-based diets on an hourly basis.

Study 1 characterised a grass silage in terms of organic matter (OM), carbohydrate and N degradability. Results were placed on a database with degradability characteristics of other feed ingredients.

In study 2, diet A was formulated from concentrate ingredients, with degradability characteristics similar to the grass silage, and supplement (S) which, when fed with grass silage (G) or A, resulted in a more synchronous ruminal hourly nutrient release. Four diets were offered: G, G+S, A or A+S, to eight growing wether lambs. G had the lowest daily intake (0.937kgDM/day) and supplementation significantly increased daily intake of G ($p \leq 0.01$) and A ($p \leq 0.01$), slightly altering intake pattern. A had a significantly different ($p \leq 0.001$) daily intake and whole tract digestibility ($p \leq 0.001$), compared to G. It was concluded that physical factors had a greater effect than chemical factors in controlling intake.

In study 3, four complete diets were formulated to further examine nutrient synchronisation, by supplementing the grass silage with concentrate: slow energy, fast N (ASYN), slow energy, slow N (INT(SE)), fast energy, fast N (INT(FE)) or fast energy, slow N (SYN).

Diets were fed *ad libitum* to four wether sheep. There was little variation between diets for daily intake or hourly intake pattern. Plasma urea levels indicated that rumen N capture was dependent on energy release supplied by the supplement, as was rumen fluid pH and osmolality, although differences were not significant ($p>0.05$). Degree of ruminal nutrient synchronisation appeared not to affect intake pattern and it was concluded that animals were unable to manipulate their intake pattern to improve synchrony.

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LIST OF ABBREVIATIONS

ADF	=	acid detergent fibre
ADL	=	acid detergent lignin
ADIN	=	acid detergent insoluble nitrogen
AFRC	=	Agricultural Food & Research Council
AOAC	=	Association of Analytical Chemists
ARC	=	Agricultural Research Council
ATP	=	adenosine triphosphate
BHB	=	beta-hydroxybutyrate
CHO	=	carbohydrate
CNS	=	central nervous system
CP	=	crude protein
DM	=	dry matter
DMI	=	dry matter
DOMD	=	digestibility of organic matter in dry matter
DUP	=	digestible undegraded protein
E	=	energy
FME	=	fermentable metabolisable energy
IOM	=	insoluble organic matter
MAFF	=	Ministry of Agriculture, Fisheries & Food
ME	=	metabolisable energy
MJ	=	megajoules
N	=	nitrogen
NDF	=	neutral detergent fibre

NH_3	=	ammonia
NH_3-N	=	ammonia nitrogen
OM	=	organic matter
RDN	=	rumen degradable nitrogen
RDP	=	rumen degradable protein
SIRE	=	Synchrony Index for the Rumen Environment
TCA	=	tri-carboxylic acid
UDP	=	undegradable dietary protein
VFA	=	volatile fatty acid
VFI	=	voluntary food intake

NS	=	$p \geq 0.05$
*	=	$p < 0.05 \geq 0.01$
**	=	$p < 0.01 \geq 0.001$
***	=	$p < 0.001$

INTRODUCTION

INTRODUCTION

Voluntary food intake is a complex subject, and the factors controlling it in the short-term are important for the initiation and termination of meals, influencing the pattern of consumption and total intake (Forbes, 1995). The meal patterns of ruminants may also affect the pattern of release of nutrients to the rumen micro-organisms and body tissues. Previous work carried out, feeding sheep at a restricted level on a diet synchronised for hourly release of energy and nitrogen, has been shown to improve microbial efficiency and reduce recycling of urea (Sinclair *et al*, 1993). However, it is not known whether ruminants will alter their meal pattern when offered a diet *ad libitum*, thus resulting in a more synchronous release of nutrients.

Grass silage is the most important winter feed for ruminants, but its intake is reduced compared to the same crop fed fresh or dry (Demarquilly, 1973). This may be due to factors such as the asynchrony of energy and nitrogen supply in the rumen and certain products of ensiling. Work has previously examined silage intake on a daily basis (Rook *et al*, 1991), but little is known about the relationship between fermentation characteristics and intake on a short-term basis.

The objectives of the current programme were to investigate the relationship between the pattern of nutrient release in the rumen and mechanisms controlling grass silage intake.

CHAPTER 1

1.1 VOLUNTARY FOOD INTAKE

1.1.1. INTRODUCTION

For maximum efficiency, animal production depends on an adequate level of voluntary food intake (VFI) (McDonald *et al*, 1988). VFI is defined as the weight of food eaten by an animal during a given period of time where it has free access to food (Forbes, 1995). The level of intake dictates rate of production, having an influence on both the biological and economic efficiency of the animal (Chamberlain and Wilkinson, 1996). By increasing an animal's VFI, there is a potential for increased production due to an increase in overall efficiency because maintenance costs are proportionately decreased as productivity increases (McDonald *et al*, 1988). However, the factors influencing intake are still not fully understood, making it difficult to manipulate VFI to the producer's advantage for more efficient livestock production.

Early theories included the chemostatic theory, in which primary blood metabolites are thought to provide the feedback information, with volatile fatty acids (VFA) being considered to be of most importance in ruminants and glucose in non-ruminants (Kennedy, 1953). The thermostatic theory proposes appetite to be linked to the animal's regulatory mechanisms (Brobeck, 1948) and the lipostatic control theory proposes that the body's fat reserves have a control on intake (Kennedy, 1953). However, no one single factor is responsible, although these theories are likely to be involved in the complex mechanism of feeding control.

Other workers, such as Baile and McLaughlin (1987) considered the hypothalamus and associated hormones to have control over the feed intake of ruminants and that the physical properties of feed in the gastrointestinal tract to only influence the rate and pattern of feeding,

the latter not being primary factors in the control of VFI.

As VFI is a widely researched subject, this review will be limited to those factors which are most important to ruminants. The most common way in which these factors are categorised is by the concept of short-term and long-term control, relating to the initiation and cessation of individual meals and maintenance of a relatively constant liveweight (in mature animals), respectively. However, these can also then be split into the following groups which will be used within this review:

i. Physical.

ii. Chemical.

iii. Physiological. (McDonald *et al*, 1988)

The factors which fall into these classes are also integrated in their mechanisms, resulting in a complex system which is not fully understood.

1.1.2 PHYSICAL FACTORS

These are factors that are linked to the physical make-up of the animal. Nervous signals relay messages from receptors in the gastro-intestinal tract back to the brain, resulting in a change in feeding behaviour (Baile, 1975).

1.1.2.1 Size Of The Reticulo-Rumen

In cattle and sheep, the reticulo-rumen contains, on average, about 75% of the gut contents, the proportion decreasing with time after feeding (Boyne *et al*, 1956). The distension of the rumen results in the stimulation of stretch and tension receptors. The bulk of many ruminant diets suggests that distension of the gut may be more important in limiting intake than in non-

ruminants (Forbes, 1985). This physical control mechanism of VFI is especially important in roughage diets, often over-riding the energy requirements of the animal (Weston, 1996). The feeding of bulky or more slowly digestible feeds, such as roughages, often provides the main bulk of ruminant diets, so this is often a problem, especially where the forage is of low feed quality (Campling *et al*, 1963).

Positive relations between the weight of the empty reticulo-rumen and VFI have been found in lambs (Wardrop and Coombe, 1960) and calves (Keslar *et al*, 1951). Calculation from data of Mäkelä (1956) showed a positive ($r = 0.63$) relationship between VFI of hay by cows and weight of the reticulo-rumen. Purser and Moir (1966) with sheep found a relationship between forage intake and rumen capacity:

$$FI = 540 + 36RC$$

where: FI = forage intake (g/day)

RC = rumen capacity (l water)

Schalk and Amadon (1928) found that removal of digesta from the reticulo-rumen stimulated intake of food, but insertion of food directly into the rumen depressed intake. In order to examine whether it is the presence of solid matter that operates this control mechanism on VFI, large bladders of water (110-220kg) were placed in the rumen for 10-14 days (Campling and Balch, 1961), and the mean VFI of hay fell by 0.54kg for every 10kg of water. They also found that pouring 220kg of water into the reticulo-rumen during the daily meal did not affect the VFI.

Krüger and Müller (1955) suggested that the cow eats to a similar fill of the reticulo-rumen at the end of a meal when offered different roughages *ad libitum*. Similarly, Blaxter *et al*

(1956) found that, at the end of a meal, the DM of the digestive tract of sheep was similar for three different roughages, suggesting that the intake was determined by the capacity of the digestive tract.

VFI is especially limited by the capacity of the reticulo-rumen when animals are fed diets containing a high proportion of roughage. Grovum (1979) cited work by Balch and Campling (1962) which reported that VFI of cows was increased when swallowed hay was removed from the reticulo-rumen through a fistula. Using the same concept, the addition of digesta, in the form of recently ingested hay, to the rumen of cows during a meal, caused an immediate decrease in intake. Similarly, inert materials such as sawdust, finely milled polyvinylchloride or polypropylene fibres (Welch, 1967) also decreased hay intake which suggests that it is the physical capacity of the rumen that is important in controlling VFI in ruminants. Similar to the findings of Campling and Balch (1961), the addition of water to rumen contents during eating was found not to affect the VFI of either cattle or sheep (Davies, 1962), the water rapidly leaving the rumen, however, when water-filled balloons of the same volume were held in the rumen intake was depressed (Balch and Campling, 1962).

In contrast to earlier observations, Baile and McLaughlin (1987) considered physical changes in the stomach, such as stretch and rate and strength of contractions to more likely be an effect of feeding than a cause of change in feeding. Other work indicates that the intestine responds to feed intake rather than contributing to the control of feed intake (Forbes, 1986). However, rate of eating and the amount eaten during a meal may be influenced by the response of the intestine to physical characteristics of the food.

These findings suggest that there is an optimum level of rumen distension which the animal will not exceed, independent of the requirement for energy. It is thought that stretch and tension receptors are therefore present in the reticulo-rumen which play an important part in controlling the intake of bulky diets but are less significant when diets are based more on concentrates. Of the compartments of the stomach, Grovum (1979) concluded that the distension of the reticulum reduced food intake the most, followed by the abomasum, and the rumen was the least likely place, despite the latter having the largest volume. In studies by Balch and Campling (1962), following the removal of water-filled balloons, intake increased gradually over a period of time. This observation could be applied to the time-lapse in increased VFI following parturition seen in ruminants - the abdominal capacity reduces rumen capacity which, in turn, reduces the intake of animals that are young, fat or in late pregnancy, whereas older and thinner animals, or those in early pregnancy, have higher intakes (Bines, 1976).

1.1.2.2 Digestibility And Rate Of Disappearance Of Food From The Gut

Rate of disappearance of digesta from the reticulo-rumen depends primarily on its rate of digestion and, ultimately, the chemical and physical composition of the food (Conrad *et al*, 1964). The less digestible the food, the longer the time spent in the rumen (Balch, 1950). Fibrous foods have a relatively low digestibility and are broken down slowly so that the food is retained in the rumen for a longer time period, as only small particles can leave the rumen (Ronning and Laben, 1966), with the cellulose content of this fibrous food being responsible for its slower digestion (Deswysen and Ellis, 1990). Therefore, there is an indirect relationship between digestibility and food consumption, linked by the rate of breakdown in the rumen. Blaxter *et al* (1961) and Campling *et al* (1961, 1962) demonstrated this inverse

relationship between retention time and VFI of roughages in both sheep and cattle.

The rate of disappearance of digesta from the digestive tract involves absorbtion of the products of digestion and fermentation of the soluble components of the feed (Forbes, 1980). However, with many diets more than 50% of the organic matter (OM) will pass undigested from the reticulo-rumen (Ketelaars and Tolkamp, 1992). Newly swallowed food is added to the fibrous mass in the dorsal rumen, so if food is available *ad libitum*, the volume remains fairly constant (Egan, 1970). Reduction of the amount of contents is also affected by microbial breakdown, by physical reduction in particle size during rumination and by onward passage to the omasum and abomasum. Disappearance of digesta from the rumen is therefore a fairly continuous process, accelerated during the intake of food (Thornton and Minson, 1973).

Campling *et al* (1961) observed that when cows were given either hay or oat straw *ad libitum* in one meal daily they ate more than twice as much hay as straw. The VFI of the two roughages was directly related to the respective rates of disappearance of digesta of each food with the slow rate of passage of the straw due to its low digestibility. In a subsequent experiment, Campling *et al* (1962) introduced urea to the rumen when feeding straw, thus increasing its digestibility by about 18% and increased the intake of the straw by 40%. Thus, it has been suggested that there is a close and probably causal relation between the extent and rate of cellulose or forage digestion and the VFI of a roughage by ruminants (Hoflund *et al*, 1948; Crampton, 1957; Huffman, 1958).

It has been well established that cattle will usually eat much more of an immature than of a

mature roughage (Graves *et al*, 1933; Forbes and Garrigus, 1950), as increasing maturity results in a decrease in the digestible energy content (Minson *et al*, 1960).

1.1.2.3 Oropharyngeal Regulation

It was once suggested that a limiting factor effect on intake may be due to fatigue of the jaw muscles or shortage of saliva (Graves *et al*, 1933; Voisin, 1952). However, Campling and Balch (1961) removed swallowed hay from the rumen of cows, via a fistula, during a meal and reported that the length of the eating period was almost doubled and the cows consumed 177% of their normal intake. They concluded that exhaustion of either the salivary glands or the muscles of the jaw was not of importance in determining the termination of a meal.

1.1.2.4 Feed Factors

The diet itself can be considered to be a physical factor, although this is really an external factor, it is likely to interact most with the physical control mechanisms regulating VFI. For example, the intake of fibrous diets is more likely to be controlled by physical mechanisms than a concentrate diet, which is more dependent on chemical mechanisms (Bines *et al*, 1969).

Altering the physical form of a feed is an effective way of manipulating food intake (Campling and Freer, 1966). Grinding a roughage increases its rate of passage through the digestive tract but reduces digestibility, especially of fibre (Forbes, 1980). Balch and Johnson (1950) suggested that the decreased digestibility of fibre was due to slower breakdown in the rumen, although other workers (Balch, 1950; Minson, 1963) have suggested it is due to the increased rate of passage of the ground feed. The process of grinding increases intake to a

greater extent with a poor roughage than with a good quality roughage (Campling, 1970).

Baile and McLaughlin (1987) found that the physical properties of the feed will also influence quantities eaten at meals and patterns of intake. For example, feeding grains resulted in large intakes in each meal with a low frequency, whilst straw resulted in small amounts consumed more frequently (Baile, 1975).

Feed ingredients are often treated in some way prior to feeding to improve their quality and digestibility. This can be done either physically or chemically, such as the chopping of forage and crushing of cereals, or treatment of grass with acids at ensiling.

Discrimination in taste by ruminants was demonstrated by Bell (1959), and differences were found between animals in their acceptance and rejection of the four classes of taste: sweet, bitter, salt and sour. Animals also avoid undesirable tastes, such as faecally-contaminated grass (MacLusky, 1960). It was found, by Forbes *et al* (1967), when ruminants are offered food without choice, the perceptive faculties, especially taste and smell, play a bigger part in the initiation of eating than in the determination of the amount eaten.

A reason suggested for the difference in VFI of individual foods by ruminants is difference in palatability (taste and texture) (Weingarten, 1993). This is often linked with the form in which the food is presented ie. chemical and physical composition, and in the case of roughages, the digestibility. Greenhalgh and Reid (1967) illustrated that changing the palatability of the food, without changing the digestibility, did have an effect on intake. Table 1.1 presents these results, where the intake of treatments 2 and 3 were significantly different

due to palatability, despite the diets having similar digestibilities.

Table 1.1 Mean values for food intake and digestibility

Treatment	Intake of organic matter (g/kg weight$^{0.75}$/day)	Digestibility of total organic matter
1. Straw	13.8	0.41
2. Straw eaten + grass through fistula	23.5	0.57
3. Grass eaten + straw through fistula	43.8	0.55
4. Dried grass	59.4	0.74
s.e.d	4.3	0.019

adapted from Greenhalgh and Reid (1967)

This was also shown more recently when Baumont *et al* (1990) observed that offering a more palatable hay could override the satiety signals due to rumen fill.

1.1.3 CHEMICAL FACTORS

This set of factors are feedback signals which involve changes or flows of specific types of substances. These operate within the gastrointestinal tract, such as changes in the pH and osmolality of digesta, and levels of volatile fatty acids in the rumen, or fluctuations of hormones and metabolites within the bloodstream (Forbes, 1988).

1.1.3.1 Volatile Fatty Acids

Volatile fatty acids (VFA) are produced by microbial fermentation in the reticulo-rumen, with the majority of the fatty acids being absorbed directly from the rumen (McDonald *et al*, 1988). They are the most important energy source for ruminants and the ratios in which they are absorbed are related to efficient ruminant production (Chamberlain and Wilkinson, 1996).

The major VFA, acetate, propionate and butyrate, are thought to have an important effect on the chemical mechanism of VFI control in ruminants (Forbes, 1986). Receptors for acetate and propionate are present in the reticulo rumen, however, butyrate is thought to be less important as it is converted to acetoacetate and ß-hyroxybutyrate (BHB) in the rumen (Grovum, 1985). Baile and Mayer (1969) injected metabolites (sodium acetate, propionate, butyrate and propionic acid) intraruminally whilst goats were eating and found that all solutions depressed intake, although butyrate was the least effective.

It was considered by Baile and Mayer (1969) that the feeding depressant effect of VFA may play a significant role in the overall regulation of energy balance since:

i. they are important energy sources to the rumen

ii. their rate of production increases with feeding

iii. they are the first and most immediate products of the digestive process to be absorbed.

The rate of production of acetate, propionate and butyrate in the rumen under fed conditions has been reported to be about 4.23, 1.17 and 0.97 mmoles/minute, respectively, for sheep (Leng and Leonard, 1965), indicating the proportions in which these primary VFA are present. However, this proportion changes, depending on the type of diet fed.

1.1.3.2 Rumen pH

Carter and Grovum (1990) considered feed intake and salivation to be important aspects of ruminal function, and that the buffers in saliva helped to maintain the pH of the rumen environment. Chemoreceptors were found, by Leek and Harding (1975), in the rumen and reticulum, the activity of which was found to change according to the pH of rumen fluid. This

has led workers, such as Forbes (1986) to conclude that the fall in rumen pH observed towards the end of a large meal to be involved in the cessation of feeding. This is related to the production of VFA, derived from carbohydrate feed sources (Ash, 1959).

Changes in rumen pH are important to the efficiency of microbial activity, as the cellulytic activity of rumen microbes decreases if the pH falls below 6.0 (Mould *et al*, 1983). Furthermore, rumen stasis was found to occur when the pH of the rumen was decreased to pH5.0 (Forbes, 1986).

Diets containing high levels of concentrates, particularly where the carbohydrate source was rapidly-degradable, were found not to be conducive to rumination, salivation or good rumen function (Carter and Grovum, 1990). This resulted in lower saliva production and a drop in pH, which consequently reduced microbial activity and a slower rate of passage of nutrients. Mould *et al* (1983) observed that feed intakes were decreased as a result.

Edwards and Poole (1983) included sodium bicarbonate (20kg/tonne) in a complete diet for dairy cows, in an attempt to maintain rumen pH following feeding. Intake was observed to increase (13.9 to 14.9kg/day) and weight loss reduced, suggesting that rumen metabolism was more efficient when a buffer was supplied within the feed.

1.1.3.3 Rumen Fluid Osmolality

In general, the osmolality of rumen contents increases during and after a meal, with Warner and Stacey (1968) reporting increases from a pre-feeding level of 250-300mosmol/kg to up to 500mosmol/kg in the few hours following a large meal. It is therefore considered that

osmolality of rumen contents is involved in the control of VFI.

In a study by Phillip *et al* (1981a), extracts from fresh and ensiled maize were adjusted with sodium chloride to osmolalities of 200-1600mosmol/kg, and ruminally infused into sheep. The reduction in food intake observed during the first 30 minutes was directly proportional to the osmolality of the infused solution

The increase in osmolality of rumen contents observed following feeding results in increases in osmolality of both digesta further down the gastro-intestinal tract and of blood. However, when this was examined by Carter and Grovum (1990), by infusions into the abomasum, little effect was seen on intake, and it was considered that osmoreceptors present in the rumen must be of primary importance.

The way in which osmotic pressure influences intake has been suggested to be due to inhibition of ruminal microfauna activity by Church (1975), whose work observed that a rumen osmotic pressure near 260mosmol/kg was most favourable for ciliate protozoan activity. Similarly, Bergen (1972) noted that cellulose degradation was inhibited *in vitro* when osmolality was greater than 400mosmol/kg.

1.1.3.4 Blood Metabolites And Hormones

1.1.3.4.a Peptides (CCK)

Opioid peptides stimulate feeding, whereas cholecystokinin (CCK) peptides reduce feeding (Baile *et al*, 1986). These two sets of peptides may interact, contributing to the overall control of VFI.

CCK is one of several peptides found in the gastrointestinal tract and subsequently in the brain, and is therefore the most likely peptide to be involved in the elicitation of satiety (Krieger, 1983). Della-Fera and Baile (1979) continuously injected CCK into the lateral cerebral ventricule of sheep, in concentrations likely to be in the physiological range, and found that feed intake decreased. Scallet *et al* (1985) found higher concentrations of CCK in fed sheep than in fasted sheep.

1.1.3.4.b Insulin

Insulin is required to induce glucose uptake in order to affect intake (Woods *et al*, 1984). The injection of insulin has been shown to stimulate feeding under some conditions in sheep, presumably by causing hypoglycaemia (Baile *et al*, 1969; Seoane *et al*, 1984). However, the role of insulin in short-term control of intake depends on the nutritional status of ruminants, as demonstrated by Faverdin (1986). Cows given 0.7µg/kg of insulin intravenously at the beginning of a meal had a decreased intake (14%) during the following 30 minutes if food had been withheld for 11 hours, but this decrease was not observed when the cows were fed 4 hours before the injection.

Other metabolites thought to be involved in the control of VFI include glucagon, growth hormone, glucocorticoids, adrenaline and neuropeptide Y. These hormones actively control the movement of energy into and out of reserve tissues (Forbes, 1995).

1.1.4 PHYSIOLOGICAL FACTORS

The products of digestion of a meal will be produced at a rate which is approximately proportional to the rate at which digested energy becomes available to the animal (McDonald

et al, 1988). The faster the rate of utilisation of substrate by the tissues, the greater the quantity of food to be eaten and digested before a satiety level is reached (Forbes, 1995), and the physiological state of an animal can alter its demand for energy (Ketelaars and Tolkamp, 1992b).

1.1.4.1 Growth

During growth, animals will consume more feed, either in anticipation of increasing energy requirements or to satisfy existing needs (Forbes, 1971). With growth comes a change in the size of the abdomen. Feed quality must therefore be high to sustain a high level of intake and, therefore, growth, such as in the form of concentrated energy (Hodgson, 1973).

1.1.4.2 Pregnancy

The pregnant ruminant has to deal with the opposing factors of increasing foetal requirements and decreasing abdominal volume (Ingvartsen *et al*, 1992).

Reid (1958) suggested that the decrease observed in twin-bearing ewes was due to the space occupied by the foetuses restricting expansion of the reticulo-rumen in the abdominal cavity. This is illustrated in Figure 1.1, where the rapid development of the foetus during the last six weeks prior to lambing can be observed, highlighting the importance of ewe nutrition during this period (Robinson *et al*, 1976).

Work by Putnam and Bond (1971) and Owen *et al* (1968) showed that cows spend less time eating during the last month of pregnancy compared to cows in early pregnancy or non-pregnant cows. Similarly, Campling (1966) observed a decrease in the intake of hay by cows

in late pregnancy compared to non-pregnant cows.

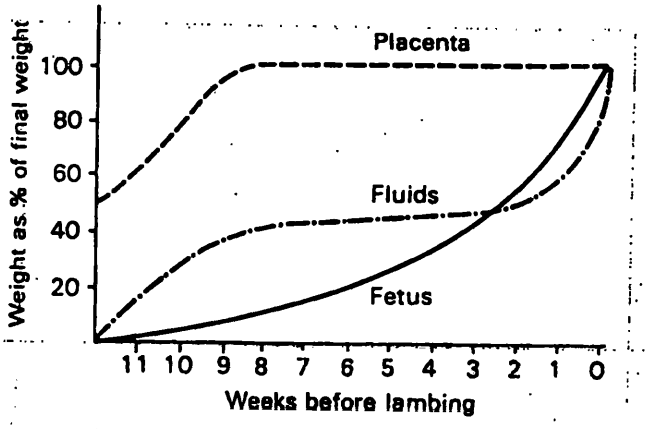

Figure 1.1 Differential developments of components of conceptus (Robinson *et al*, 1978)

1.1.4.3 Lactation

Daily intake is the most common limiting factor in sustaining milk production (Chamberlain and Wilkinson, 1996). Energy intake increases at the onset of lactation, with the demand for milk synthesis, although this increase generally lags several weeks behind increase in milk yield (Journet and Remond, 1976). These events can be observed in Figure 1.2 (Webster, 1987). In cows and sheep, milk production is often so great that with normal systems of feeding the animals lose weight during the first few months of lactation (Reid, 1961), but this is usually by cows in later lactation as VFI remains high, whereas in sheep the lambs will be weaned and the ewe dried off.

Elliott *et al* (1961) and Cook *et al* (1961) estimated that the intake of herbage by lactating cows and ewes, compared to non-lactating animals grazing the same pasture, are about 13 and 24% greater, respectively.

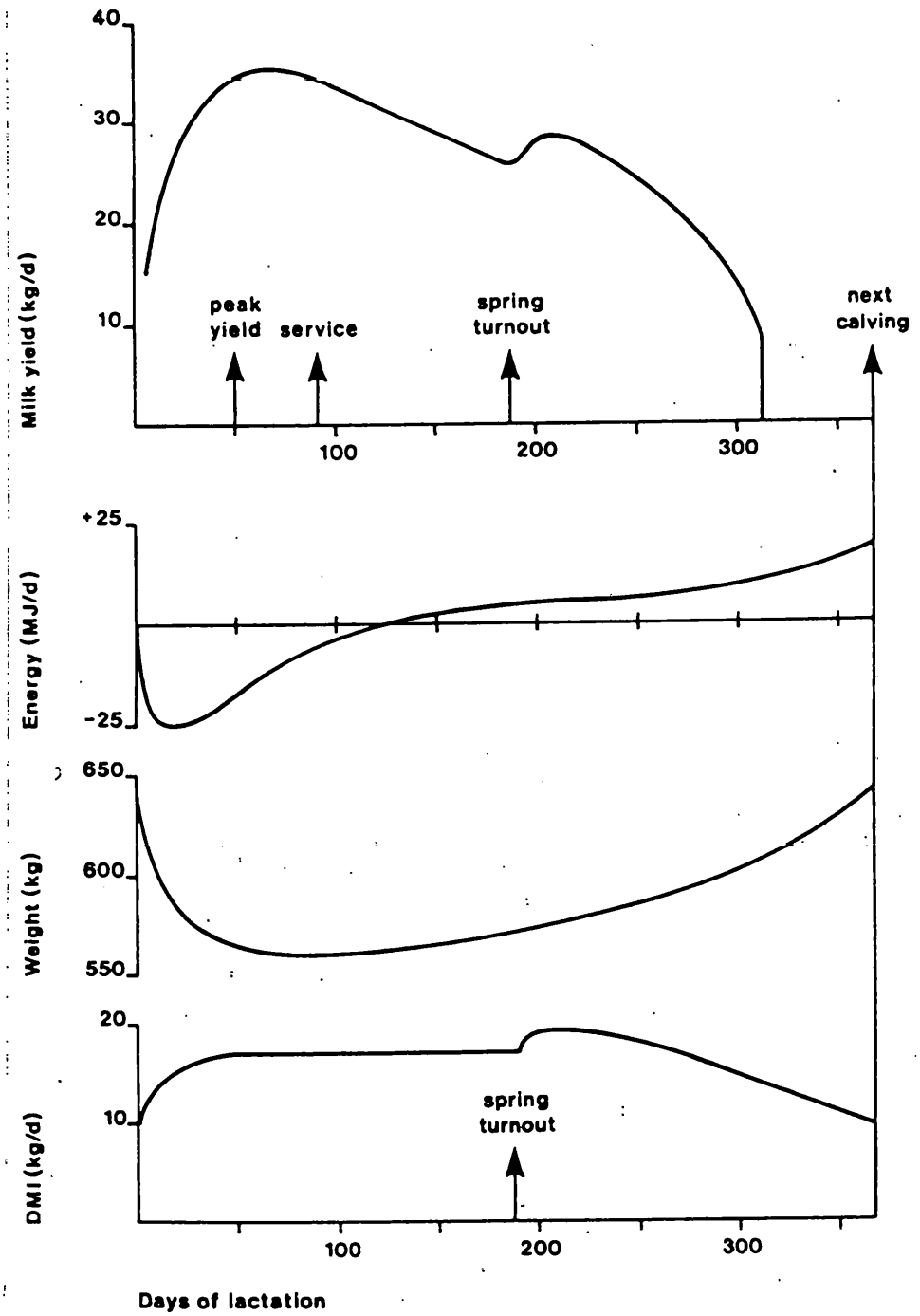

Figure 1.2 The sequence of events during lactation for a typical autumn-calving Friesian cow (Webster, 1987)

1.1.4.4 Fat Animals / Degree of Fatness

In fat animals, a substantial proportion of energy is stored in the form of adipose tissue, a varying amount of which is stored within the abdominal cavity and is therefore competing with digesta for abdominal space. Half of any change in body fat has been assumed to be intra-abdominal (Russel *et al*, 1971), and this in turn displaces half of its weight of gut contents. It is generally known that adult cattle and sheep eat less when fat than when thin (Mather, 1959; Ferguson, 1956). This was demonstrated by Bines *et al* (1969) when the intakes of forages by fat and thin cows was compared, and results are presented in Table 1.2.

Table 1.2 Mean daily voluntary intakes of straw, hay and hay plus concentrates by six cows when fat and thin

	Total daily intake of DM (g/kg liveweight$^{0.75}$)		
Diet	Fat	Thin	Difference (thin - fat)
Straw	30.8	38.1	7.3
Hay	50.9	89.7	38.8**
Hay plus concentrates	74.1	112.6	38.5**
s.e.d.	4.96		

** significant (p<0.01)

Bines *et al* (1969)

As body fatness has such a pronounced effect on VFI, attaining appropriate body condition targets in the cycle of pregnant and lactating animals is important (Webster, 1987).

1.1.4.5 Heat/Thermostatic Control

Heat stress causes reduced feed intake and general performance, and a negative energy balance may result when the climatic temperature reaches 40°C (Appleman and Delouche, 1958; Ragsdale *et al*, 1950). Under severe cold conditions, where heat loss from the animal is increased, feed intake increases and the efficiency of production is reduced (Moose *et al*,

1969). Increases in gut motility and rumen outflow rate were observed under cold environmental conditions by Christopherson and Kennedy (1983), providing an explanation for increases in food intake.

Internal temperature of the ruminant is also important. Gengler *et al* (1970) increased the temperature of rumen contents from the normal 38.0°C to 41.3°C, with heating coils in the rumen, and depressed intake by 15%. Similarly, reducing the temperature of the rumen of cattle by adding cold water of 5°C led to a decrease in body temperature of 0.5°C and an increase in intake of 24% (Baile and Forbes, 1974).

1.1.4.6 Energostasis

Farm animals control their intake primarily by monitoring their consumption of available energy. Forbes (1980) proposes that 'feeding starts in response to a lack of absorbed energy-yielding substrates, relative to energy requirements and terminates when there is a relative excess of energy, or when the gut is full'. This is based on the concept of 'energostasis'.

Trials by Cooper *et al* (1995) have shown that animals offered feeds varying in their energy concentration altered their DM consumption by altering meal size or frequency to achieve an energy balance. The mechanisms of energostasis are not fully understood, although it is known that the hypothalamus is involved in receiving neural and endocrine information which regulate food intake (Baile and McLaughlin, 1987).

A variety of feedback signals provide the centres of the brain with the control of energostasis and consequently food intake, including blood metabolites, metabolic hormones (eg. growth

hormone, insulin and glucagon) and gut peptides (eg. cholecystokinin) (Forbes, 1988).

Although energostasis is considered to be the underlying mechanism in VFI, there are factors which limit food intake physically. The capacity of the digestive tract, the rates of digestion and absorbtion and the rate of passage through the tract will all affect VFI and limit the animal's ability to achieve energostasis (Booth, 1978). The range of energy concentrations over which energostasis can be accomplished will also be dependent on the physiological state of the animal, its social environment and the characteristics of the feedstuff (Egan, 1970).

1.1.4.7 Competing Drives / Availability Of Food

Freer *et al* (1962) found that when the time of access to roughages was limited, the rate of eating was increased. However, intake was greater when animals had continuous access than with a limitation of 4.5 hours daily with a difference of 21% with hay and greater differences with concentrate diets.

1.1.5 FACTORS AFFECTING VFI OF GRASS SILAGE

1.1.5.1 Introduction

Forages are often limited in their potential to contribute to the total nutrient supply to a ruminant, owing to their relatively low intake (Heaney, 1968). Grass silage is especially limited in its potential and it is well established that its DMI is significantly less than that of the same crop fed fresh or as hay, for example, Demarquilly (1973) found a mean reduction of 33% in sheep when grass silage was fed, compared with the same material fed fresh. A selection of these results are presented in Table 1.3. ARC (1980) found this reduction to be

greater in cattle than in sheep. No single factor has been attributed to this low intake, although the major factors are considered to be low DM, bulky nature of the feed, slow rates of degradation, low pH, presence of protein breakdown products and nutrient imbalances (Chamberlain and Wilkinson, 1996).

Table 1.3 Relative ingestibility by sheep of grass silages in comparison to fresh herbages

Harvesting machine	Method of preservation	n	DM intake	
			g DM/kg $W^{0.75}$	$\frac{\text{Silage}}{\text{Fresh herbage}} \times 100$
Precision-chop (0.5-1.5cm)	Direct cut without additive	36	53.4	79
	Direct cut with efficient additive	64	56.0	83
	Direct cut with formic acid and formaldehyde	21	51.8	77
Precision-chop and double-chop (2-5cm)	Direct cut without additive	20	41.0	64
	Direct cut with additive	27	46.0	71
	Wilted	11	44.5	67

adapted from Demarquilly (1973)

However, these factors contributing to low grass silage intake are usually specific to particular situations. Rook *et al* (1991) assessed a number of feed and animal factors and found that the most important variable affecting silage intake in lactating dairy cows were, in order of importance, silage NH_3-N, milkfat yield, concentrate DMI, digestibility of the organic matter of the silage DM (DOMD) and animal liveweight.

1.1.5.2 Dry Matter Content

The relatively low DM (20-30%) of grass silage as a feed is probably the primary factor resulting in its low intake (Thomas *et al*, 1961) and there is a positive correlation between silage DM and VFI (Castle and Watson, 1984). The intake of wilted silage is generally higher

than that of unwilted silage made from the same crop (Raymond *et al*, 1986) as, even if an unwilted silage is well-preserved, it will contain more acids. However, nutrient losses do occur during this stage of silage-making, and this must be taken into account when assessing the benefits of wilting (Thomas and Thomas, 1988). A number of reports show higher intakes for wilted compared to unwilted silages (Marsh, 1979; Bertilsson, 1987) and more recently by Fitzgerald (1996a), who found wilting slightly more advantageous in increasing intake on longer chopped and more mature crops.

However, the increase in intake is not always reflected in animal performance, for example, Martinsson (1992) found no significant effect on milk yield for wilted silage.

1.1.5.3 Physical Limitations / Bulkiness of Forage

The amount of grass silage that a ruminant can intake is likely to include physical constraints due to the bulky nature of the feedstuff. Intake of silage is often, but not always, related to its digestibility, usually as a positive correlation (Harris and Raymond, 1964), although this is illustrated more clearly if a wide range of digestibilities are examined (Murdoch, 1965). A low digestibility feed, such as a forage, occupies space in the rumen for longer, thus limiting intake, which also relates to physical fill of the rumen. Balch and Campling (1961) found that when bags filled with 16l of water were placed into the rumens of dry cows, that silage intake decreased by 16%, and Farhan and Thomas (1978), who inserted water-filled bags into the rumen and removed digesta from the rumen, found VFI was consistent with appetite being controlled through a rumen-fill mechanism.

Reducing particle size of the grass by chopping during the silage-making process will increase

the density of the feed and the surface area available to rumen micro-organisms, increasing its rate of passage through the digestive tract so that VFI is increased. This has been shown in both dairy cows (Murdoch, 1965; Dulphy and Demarquilly, 1973) and beef cattle (Wilkinson *et al*, 1978).

1.1.5.4 Chop Length

Chopping grass prior to ensiling generally increases intake due to an improved fermentation in the silo and an increased rate of passage through the digestive tract (Castle *et al*, 1981). In nine comparisons using sheep, mean intake increased by 56% for silages which were precision chopped, compared to silages which were flail harvested (McDonald *et al*, 1991). This chopping prior to harvesting was also found to be more effective in increasing VFI than chopping prior to feeding.

Fitzgerald (1996b) carried out a series of trials examining the effect of chop length on VFI and performance of store lambs. Grass harvested with a double-chop harvester had a longer rumen retention time (21.4-29.3 hours) than grass harvested with a precision-chop harvester (12.6-20.6 hours) which, in turn, increased VFI by 39-49%.

Wilkinson *et al* (1978) found an increase in VFI in young beef cattle of 66% for grass silage chopped to 8mm compared to a 33mm chop length. This may have been influenced by the greater digestibility of the 8mm chopped silage. A trial by Deswysen *et al* (1978) compared grass silage chopped to either 53mm or 18mm before ensiling and grass silage chopped to 18mm prior to feeding. Sheep offered the long silage had lower intakes and spent less time ruminating. There was no difference between either 18mm silage, and pseudo-rumination was

significantly increased on the longer silage, suggesting the animals had problems in regurgitating.

Production levels can also be improved by offering chopped material, partly due to the increase in VFI. The intake of a perennial ryegrass silage and the milk yield of cows increased as chop length decreased in a trial reported by Castle *et al* (1979). Time spent eating and ruminating decreased as chop length was reduced, but the mean retention time of food particles in the digestive tract was not affected.

1.1.5.5 Digestibility / Maturity of Herbage Ensiled

It has long been established that the grass silage that results in the highest animal production is made from rapidly growing grass cut at the leafy stage of growth when the DOMD, or D-value, is greatest (Dodsworth and Campbell, 1952, 1953). As grass digestibility decreases, VFI will decrease (Raymond *et al*, 1986) and nutrient intake is largely determined by the product of intake and digestibility. The digestibility of a forage is further increased by ensiling (Keady *et al*, 1995). Animal production is impaired as the quality of forage is decreased by the development of grass plants during the growing season (Castle, 1982; Steen, 1992). This is due to the proportion of cell walls increasing in the plant and increased lignin content in cell wall material (Jung, 1989). It is therefore important to harvest the crop at the time which will achieve the best herbage for ensiling. Another factor to consider is that in mature plants, N content is lower, but the efficiency of capture of N in the rumen of N-rich silages harvested at an early stage of growth is relatively poor (Tamminga, 1992). Rinne *et al* (1997) noted that as the plant matures and digestibility increases, in turn there follows a decrease in digestible energy content, N content and available minerals, as well as a low intake potential.

However, later harvesting increased DM yield and minimised N losses from the field and animal. In a second study, Rinne *et al* (1997) took four harvests from the same sward at approximately weekly intervals. Results showed that as the grass matured, OM decreased, rumen pH increased, rumen NH_3-N concentration decreased and acetate:butyrate proportion in the rumen increased. Additionally, N intake decreased but duodenal NH_3-N decreased, suggesting greater rumen losses. The efficiency of microbial protein synthesis was not affected by maturity but both the apparent N digestibility and degradability of N in the rumen decreased.

Improvements in production level were observed when Fitzgerald (1996a) compared grass silages cut at either a leafy or a more mature stage of growth. It was found that intakes were increased (650-667g DM/day compared to 534-589g DM/day) and the less mature, more digestible grass maintained lamb liveweights, whilst VFI of the silage produced from the more mature grass resulted in a loss in lamb liveweight.

1.1.5.6 Fermentation Pattern / Breakdown Products

During the silage-making process, soluble carbohydrates are fermented, producing organic acids (McDonald and Edwards, 1976). For production of a good quality silage, lactic acid must dominate the fermentation (Murdoch, 1989). However, acetic acid and nitrogenous compounds, such as amines, are also produced. These fermentation products, in combination, are thought to have a part in decreasing the VFI of silages. These factors have been reviewed by Dulphy (1977) and Waldo (1977).

In a poorly fermented silage, where undesirable micro-organisms are present, breakdown

products from proteins produced by the bacteria can limit intake (Flynn, 1988). Even in the best preserved silages, about 60% of CP of grass is degraded to peptides and amino acids (Forbes, 1995). Ammonia (NH_3) content is highest in a silage where the proteins have been broken down, and Tayler and Wilkins (1976) showed that the presence of NH_3 and VFA (especially acetic acid) will decrease VFI. NH_3-N is an indicator of clostridial activity in silage and is used as an indicator of fermentation quality in laboratory analysis (Chamberlain and Wilkinson, 1996). *Clostridium spp.* can ferment carbohydrates and lactic acid to form butyric acid which has a rancid smell and is unpalatable to stock (Tayler and Wilkins, 1976). These bacteria are most commonly introduced by soil contamination during mowing and from machinery during compression of cut grass in the silo. *Clostridium sporogenes* can break down amino acids to ammonia and amines, some of the latter being toxic to stock.

The effects that fermentation products of silage have on VFI have been examined in a number of ways. Phillip *et al* (1981b) infused nitrogenous extracts from maize silage into the rumen of hay-fed animals and found a decrease in intake of the hay offered. This effect, that silage extract had on intake, was also highlighted in a number of trials, including those reported by Buchanan-Smith and Phillip (1986), who intraruminally infused sheep with isosmotic saline, organic acids or lucerne silage extracts, with or without other acids and amines. They found that those infused with the silage extracts significantly decreased their intakes 4 hours after feeding, compared to sheep not infused. Clancy *et al* (1977) studied the difference in hay intake between intraruminally infusing either silage juice or a synthetic mixture containing the same VFA, lactate, soluble carbohydrates, NH_3 and nitrate, which had the same pH and osmolality as the silage juice. Although both treatments resulted in reduced VFI, the intakes of animals infused with the silage juice were 40% lower than those infused with the synthetic

mixture. This suggests that the amines and compounds which were not present in the synthetic mixture must have an important effect.

McKee *et al*, (1996) examined partially replacing grass silage with fresh grass on rumen fermentation characteristics and rumen outflow rates in cattle. It was found that rumen NH_3 concentration was reduced, as well as the proportion of rumen propionate, *i*-butyrate and *n*-valerate. There was an increase in the proportion of rumen acetate and in both the particulate and liquid outflow rates from the rumen. This suggests a reason for improvements seen in animal performance when a grass silage is supplemented with fresh grass.

Nitrogenous compounds, including amines, are formed during fermentation as a result of decarboxylation of amino acids (McDonald *et al*, 1991). Dawson and Mayne (1995) intraruminally infused the amines putrescine and cadaverine, but found no significant effect on the intake of grass silage. However, van Os *et al* (1995) found that adding NH_3 to grass silage did not alter VFI, whereas amines slightly lowered VFI. This was thought to be due to their effect at the oro-pharyngeal level of control. Gill *et al* (1988) also suggested that the fermentation products present in silage may regulate intake by oropharyngeal properties or by metabolic means. This is in accordance with observations by Greenhalgh and Reid (1967) who suggested VFI is regulated by a combination of palatability and post-ingestive feedback mechanisms.

VFA are another product of the fermentation process and, although the presence of lactic acid as the dominant VFA is necessary for a successful preservation, presence of other, less favourable VFA can reduce VFI. Thomas and Chamberlain (1983) found a positive

correlation between intake and the ratio of lactic acid:total acid, whilst the presence of high concentrations of short-chain VFA, particularly acetic acid, was found by Wilkins *et al* (1971) and Demarquilly (1973) to decrease the intake of well-preserved silage.

Thomas *et al* (1980) suggested that chemicals in silage may act through physical mechanisms and this interaction was evidenced by Smith and Clapperton, 1981. Lucerne silage juice was intraruminally infused, which resulted in a depression in rumen motility and thus the rate of eating also decreased.

1.1.5.7 Acidity

There is a limit to the amount of acid that animals can intake (Raymond *et al*, 1986). However, the main aim of the ensilage process is to decrease pH. Both Wilkins *et al* (1971) and Brown and Radcliffe (1972) found that the intake of a well-preserved silage was limited by low pH. However, Farhan and Thomas (1978) suggested that pH is not a prime factor limiting appetite after finding no significant improvements in silage intake with bicarbonate-treated silage.

It has been proposed, due to the relationship between intake and pH, that partial neutralisation with bicarbonate may increase intake and this was found to be the case by McLeod *et al* (1970). Thomas and Chamberlain (1983) found inconsistent results in calves, whereas, Farhan and Thomas (1978) found adding bicarbonate to grass silage decreased intake in cattle and had little effect in sheep, as well as decreasing rumen NH_3 levels.

1.1.5.8 Additives

There are many additives available on the market, generally classified to either improve fermentation, increase intake, or both.

Formic acid is often used as a relatively cheap silage additive. This results in a lower pH than that of a control silage, and the formic acid reduces the protein breakdown in the clamp due to a rapid fall in pH early in the ensilage process. Secondary fermentation is prevented and the production of VFA and NH_3 is decreased, resulting in a good quality silage. However, VFI has been shown to be lower in some of these silages (Castle and Thomas, 1975). This limitation has also been shown to be reduced to some extent by supplementing with a high protein concentrate (McLeod *et al*, 1970; Thomas *et al*, 1980).

The addition of bacterial inoculants have given variable results, but are capable of increasing intakes compared to some acid additives, even when the grass silages are similar in their chemical composition (Mayne, 1990). Keady and Murphy (1993) and Smith *et al* (1993) found that the inoculant treatment of herbage at ensiling does not always result in improvements in animal performance, perhaps due to factors such as differences in the inoculant bacteria species or strains and concentrations of bacteria present. More recently, Keady *et al* (1996) concluded that, relative to a well-preserved untreated silage, using a bacterial inoculant additive did not alter DMI, milk yield or fat plus protein yield when grass silage was offered to lactating cows. However, milk protein concentration was found to increase.

Mayne (1993) made comparisons between using formic acid, sulphuric acid and bacterial

inoculation as additives during silage-making. It was found that in silages made from a first regrowth that the addition of formic acid resulted in a 12% increase in VFI, bacterial inoculation increased VFI by 4.6%, but sulphuric acid decreased VFI. No significant effects were found by using the same additives to grass silage produced from a second regrowth.

1.1.5.9 Supplementation With Concentrates

Silage cannot usually be eaten in sufficient amounts to satisfy nutrient requirements for productive growth or milk yield and it therefore requires supplementation (Moore *et al*, 1999).

Providing a source of readily-fermentable carbohydrate can result in a decreased digestibility of the grass silage due to a drop in rumen pH and subsequent decreased rate of cellulose digestion, in turn decreasing silage intake (Thomas *et al*, 1986). The rate at which fibre is digested is also reduced (Raymond *et al*, 1986), so that when a cereal-based supplement is fed, the rate of passage of particles of forage through the rumen decreases. The overall result means that a cereal supplement partly replaces, or substitutes, rather than fully supplements the forage with which it is fed.

The concept of substitution rate has long been established. A study by Murdoch (1964) involved supplementing *ad libitum* hay or grass silage diets of young sheep with barley at levels of 0 to 750g/day. On average, the ingestion of 1 unit of barley reduced the DMI of hay by 0.64 units and of silage by 0.26 units.

Castle and Watson (1976) found a substitution rate of 0.51 when barley was added to a silage

diet for Ayrshire cows. However, they also found that the level of production was often higher when a highly digestible supplement, such as barley, was provided. More recently, Fitzgerald (1996b) supplemented grass silage with barley in sheep diets and reported a reduced silage intake of 9% compared to silage fed alone. However, total DMI was increased by 30%, and ME intake by 37%. Substitution rates averaged 0.24g silage DM/g barley DM. Supplementation with barley resulted in improved liveweight gains (28-93g/day for silage only compared to 90-150g/day for silage supplemented with barley) (Fitzgerald, 1996b).

When dried grass cubes were used as a supplement to grass silage, the substitution rate decreased to 0.36 compared to 0.51 for barley. Similarly, molassed beet pulp resulted in a substitution rate of 0.4 (Castle and Watson, 1979). These results suggest that the more similar the supplement to the chemical composition of grass silage, the lower the substitution rate.

1.1.5.10 Nutrient Release Pattern / Asynchrony of nutrients

Despite the nutrient content of grass silage appearing to be adequate for ruminant diets, the way in which these nutrients are available may also result in limiting factors in terms of the rumen (Raymond, *et al* 1986). During the ensiling process, approximately 10% of the DM of grass ensiled disappears due to the fermentation of soluble carbohydrates. This is equivalent to half the potential energy (in the form of ATP) supplied to the rumen, which consequently results in poor microbial growth (Forbes, 1995). This restriction in the growth and activity of micro-organisms means that fermentation and particle breakdown for grass silage are lower than those feeds with higher contents of soluble carbohydrates, thus decreasing VFI to some extent.

1.2 RUMEN METABOLISM

1.2.1 INTRODUCTION

The dietary nutrients present in any animal's food must be modified in order to make them available for absorption (Rook and Thomas, 1983). In the ruminant, this is achieved by the micro-organisms present in the rumen that live in symbiotic association with the animal, which allow the rumen to have a major influence on the control of energy and N nutrition of ruminants (Webster, 1987). The microbes break down carbohydrates and nitrogenous compounds in the diet and convert them into utilisable products. Some fermentation products can be absorbed directly across the rumen wall, whilst others pass into the lower intestinal tract, where absorption occurs or further digestion prior to absorbtion (Shirley, 1986).

Within the rumen, there are three main groups of micro-organisms providing enzymes for fermentation; bacteria, fungi and protozoa (Hungate, 1966). The relative numbers of different species vary with the composition and structure of the feed and interactions between the groups are complex. The proportions of the different end-products vary, although these normally consist of carbon dioxide, methane, volatile fatty acids (VFA) and ammonia. A generalised view of energy and protein digestion in the rumen is presented in Figure 1.3 (Webster, 1987).

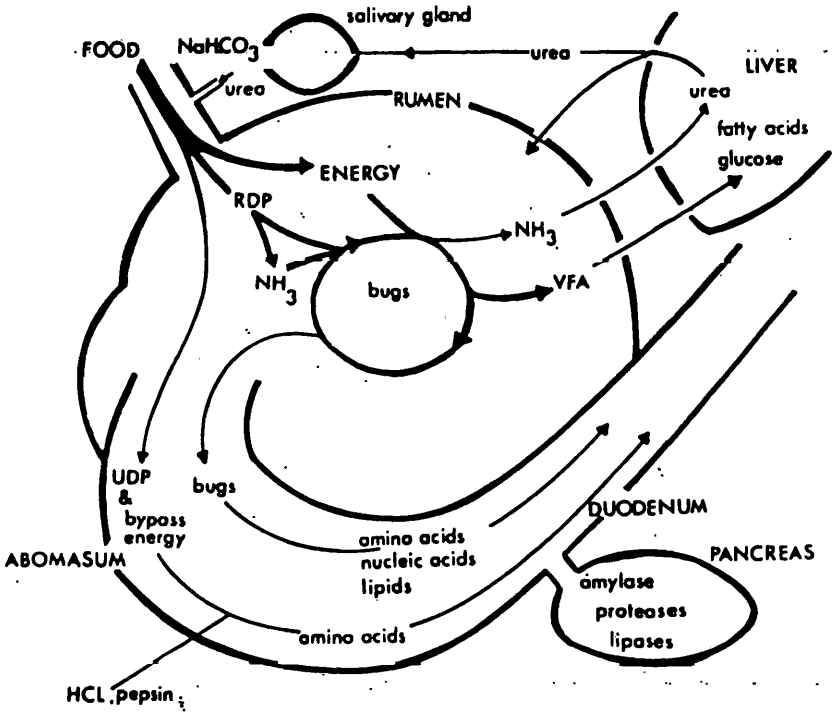

Figure 1.3 Digestion of energy and protein in the rumen and abomasum.
RDP = rumen degradable protein, UDP = undegradable dietary protein
(Webster, 1987)

1.2.2 ENERGY METABOLISM

Dietary carbohydrates are subject to fermentable degradation in the rumen, but only energy which can be utilised from the feed carbohydrate source anaerobically is available to the micro-organisms. The end-products of this process are VFA, mainly acetic, propionic and butyric acid, as well as carbon dioxide and methane. The production of VFA represents up to three-quarters of the effective energy value of the diet (Blaxter, 1962).

The different types of carbohydrates provided by the feed have varying rates of degradation, depending on their structure, the most rapidly degradable being soluble carbohydrates, which are degraded more quickly than starch, with the cell wall carbohydrates being the slowest to degrade in the rumen. Chesson (1990) classified and described these three forms of carbohydrates as:

i. Water-soluble carbohydrates, consisting largely of simple sugars and some soluble polysaccarides, such as fructosans

ii. Starch, an α-glucan-based storage polysaccharide

iii. Structural polysaccharides contributing to the plant cell wall

Figure 1.4 (McDonald *et al*, 1988) illustrates the breakdown of these carbohydrates in the rumen.

Both rate of degradation and rate of absorption will determine the concentration of VFA in the rumen. VFA are absorbed without active transport in their free form. The production of these acids lowers pH in the rumen, but this is neutralised by buffers present in saliva (Mould and Ørskov, 1984).

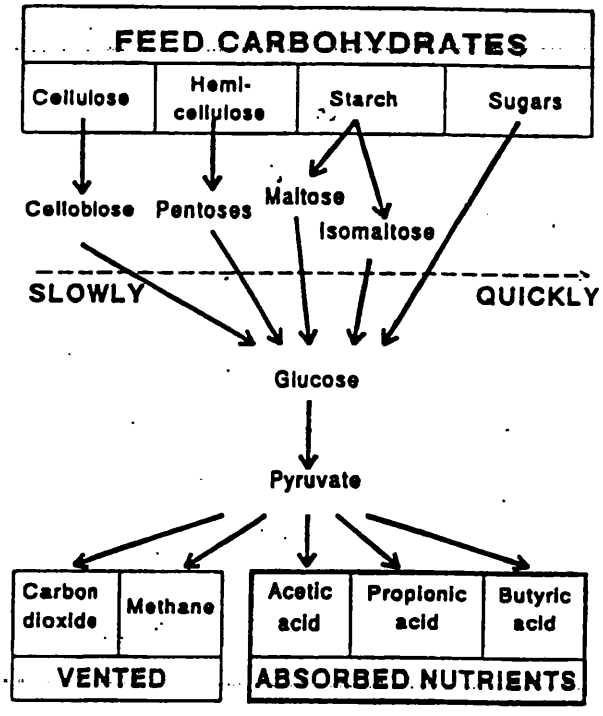

Figure 1.4 The breakdown of carbohydrates in the rumen (adapted from McDonald *et al*, 1988)

The proportions of VFA produced by carbohydrate fermentation will depend on the type of diet fed. The ratio of the major VFA is particularly important to the efficient production of ruminant animals, therefore, dietary formulation attempts to manipulate this ratio to improve ruminant production (Ørskov, 1975). These proportions are a function of the microbes present in the rumen because different microbial species use different nutrients as their principal substrates. Therefore, the VFA produced will depend on the presence and maintenance of a specific species of microbe (Ørskov, 1990).

Sugars and other water-soluble carbohydrates are rapidly degraded in the rumen, resulting in high overall concentrations of VFA. This is due to these compounds being utilised by many different types of rumen micro-organism (Hungate, 1966).

Starch-fermenting micro-organisms produce relatively more propionic acid from degradation of this type of feed source, compared to other VFA produced, although this is affected by rumen pH (Ørskov *et al*, 1974). The variation in pH will depend on the form of the feed offered, and starch concentrate sources are often processed, either physically or chemically, prior to feeding (Ørskov *et al*, 1978).

Cellulolytic micro-organisms are responsible for the fermentation of fibre (Blaxter *et al*, 1962) which results in a greater proportion of acetic acid within the total VFA produced.

Some fermentable carbohydrates may escape rumen degradation, particularly structural carbohydrate which is lignified, preventing microbial degradation. A limited amount of degradation may occur in the caecum, supplying a further source of VFA, but the majority

of this material is excreted in the faeces.

The rate of absorption of VFA will vary according to feeding regime and the fermentability of carbohydrate sources (Ørskov, 1966). For example, when animals are fed on a forage diet, carbohydrates are provided mainly in the form of structural polysaccarides such as cellulose and hemicellulose, and water-soluble carbohydrates make a lesser contribution (Czerkawski, 1986). This type of diet is therefore usually supplemented with a starch-based concentrate, such as a cereal, to attempt to improve the fermentation and availability of the nutrients supplied.

VFA are absorbed across the rumen wall, down a concentration gradient, the rate of absorption therefore being dependent on the rate of production in the rumen, although other factors do have an influence (Armstrong *et al*, 1957). Acetic acid is the main VFA absorbed and is converted to energy via the TCA cycle. If the amount of acetic acid absorbed is excess to requirements, the surplus is synthesised into long-chain fatty acids and stored in adipose tissue. Alternatively, butyric acid can be used as an energy source, following conversion to 3-hydroxybutyrate (Sutton, 1980).

Methane is the main gas produced by fermentation of carbohydrates, accounting for a loss of approximately 7% of food energy entering the rumen (Shirley, 1986).

1.2.3 PROTEIN METABOLISM

Protein degraded in the rumen is termed rumen degradable protein (RDP). This is hydrolysed by microbes to yield free amino acids and accounts for approximately 70% of protein

supplied (McDonald *et al*, 1988). However, the remainder tends to resist breakdown and is termed digestible undegradable protein (DUP). Figure 1.5 (Chamberlain and Wilkinson, 1995) illustrates the sites of degradation and digestion of protein in the ruminant.

Dietary RDP is initially hydrolysed by bacterial processes within the rumen to yield free amino acids by the 30-50% of ruminal bacteria which are considered to be proteolytic (Prins *et al*, 1983). These amino acids are either utilised by the rumen micro-organisms to synthesise microbial protein or degraded to produce ammonia (NH_3), organic acids and carbon dioxide (Asplund, 1994), the NH_3 produced providing the main source of N for the synthesis of microbial protein.

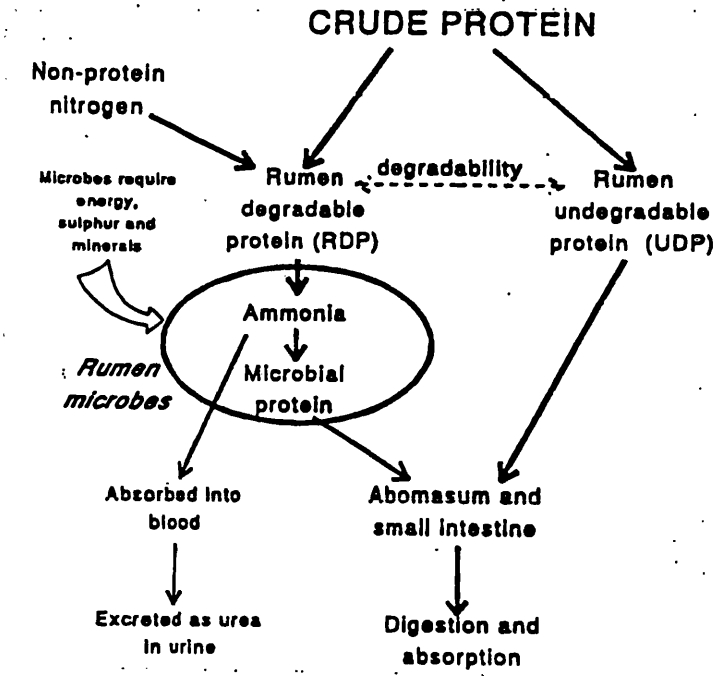

Figure 1.5 Digestion of protein in the ruminant (Chamberlain and Wilkinson, 1995)

The rate of microbial protein synthesis and the efficiency with which N is converted to microbial protein is dependent on energy available in the rumen. Approximately 7.8g of microbial protein is synthesised / MJ ME when NH_3 is not limited (ARC, 1980).

If the rate of NH_3 production exceeds the rate of utilisation, NH_3 concentrations increase in the rumen, resulting in the absorbtion of NH_3 into the blood (Smith, 1979). This is converted to urea in the liver and excreted in the urine, although approximately 20% is recycled to the rumen via the saliva (Nolan *et al*, 1973). Smith (1979) therefore proposed that, to achieve maximum feed efficiency, the diet should supply the rumen with a N source that results in the minimum concentration of NH_3 needed to maintain an adequate supply within the rumen bacterial cells.

Requirements of NH_3 by rumen microorganisms can be derived from non-protein N sources, such as urea, which is rapidly hydrolysed by bacterial ureases to yield NH_3 (Loosli and McDonald, 1968). Where urea is supplied, there must be sufficient readily-fermentable carbohydrate supplied to the rumen in order to achieve efficient conversion of NH_3 into microbial protein, as feed ingredients supplying non-protein N are often expensive (Chamberlain and Wilkinson, 1995).

Microbial protein produced by ruminal biosynthesis passes to the abomasum, and consequently the small intestine, in the form of micro-organisms within the rumen fluid (when they become detached from food particles in the rumen) together with DUP. These proteins are then digested and absorbed (Cottle, 1980).

Amino acids which result from the digestion of dietary protein are absorbed from the small intestine into the portal blood, where they contribute to the metabolic pool of amino acids in the blood. These are then used to build nitrogenous substances such as muscle or milk proteins (Harvey, 1970). The influence of the diet is still important at this stage, as the proportions of amino acids in the metabolic pool in the blood are influenced by the profile of amino acids absorbed from the small intestine which, in turn, is dependent on the composition of microbial protein produced from dietary RDP and DUP, as studied by Bergen *et al* (1968). Different diets are likely to have different amino acids limiting, for example, methionine tends to be limiting in grass silage-based diets (Asplund, 1994). This is where the formulation of the diet is important, by the inclusion of DUP of appropriate amino acid composition or synthetic amino acids, in order to manipulate this balance, as reported by Erasmus *et al* (1992) where the influence of manipulation of amino acid proportions on the rumen fermentation and duodenal N flow of dairy cows was studied.

1.2.4 ENERGY & NITROGEN SUPPLY TO THE RUMEN

1.2.4.1 Introduction

Microbes must be supplied with sufficient material in the correct proportion to enable successful growth (Oldham *et al*, 1977). If this is achieved, then rumen fermentation can potentially supply 70-100% of the ruminant's supply of amino acids (AFRC, 1992) and 70-85% of the animals's energy supply can be absorbed as VFA (Dewhurst *et al*, 1986), which are the main end-product of microbial fermentation of the carbohydrate fraction of a feed.

Current systems for the formulation of diets for ruminants are based on the daily supply of energy to the rumen, expressed in terms of carbohydrate (Madsen, 1985), fermentable

metabolisable energy (FME) (AFRC, 1992) or organic matter (OM) (ARC, 1984; Vérité and Peyraud, 1989), or by the supply of nitrogen (N) to the rumen in the form of rumen degradable protein (RDP) (ARC, 1980).

1.2.4.2 Nutrient Supply To The Rumen

1.2.4.2.a Carbohydrates

Johnson (1976) classified carbohydrates on the basis of rate of rumen degradation, resulting in three classes:

i. simple sugars - rapidly absorbed

ii. readily-fermented forms of glucose polymers such as starch and dextrins - intermediate release

iii. cell wall CHO such as cellulose and hemicellulose - slowly released

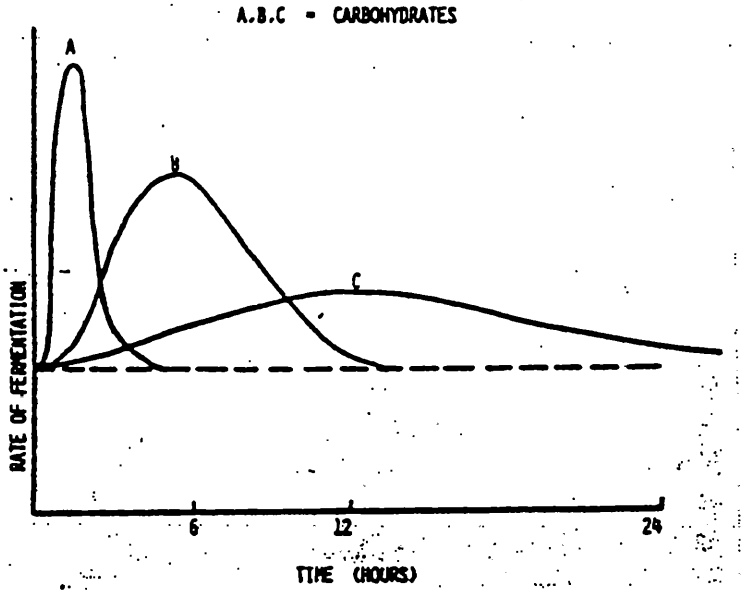

Figure 1.6 Theoretical rumen fermentation rates over time after ingestion of three forms of feed carbohydrate
A = soluble sugars, B = starches and dextrins, C = cell wall carbohydrates
(Johnson, 1976)

Figure 1.6 illustrates the fermentative activity of these three classes of carbohydrates. It can be seen from Figure 1.6 that both the amplitude of the fermentative curve and the time span varies between the various classes.

1.2.4.2.b Nitrogen

The majority of rumen microorganisms utilise N which has passed through the NH_3 pool in the rumen, with 60-80% of N passing through this NH_3 pool (Johnson, 1976). Thomas (1973) and Blackburn (1965) suggested that the amount of rumen NH_3 required to support protein synthesis was similar for each type of protein supply, in terms of rate of degradation, to that proposed for carbohydrates. This is illustrated in Figure 1.7.

This concept, proposed by Thomas (1973) and Blackburn (1965), makes the assumption that the groups of microorganisms which utilise the three classes of carbohydrate have the same ability or requirement to utilise N passed through the NH_3 pool as the major source of N. Most cellulose digesting micro-organisms can utilise NH_3 as their major N source (Bryant, 1973), as can the starch-digesting species (Hungate, 1966). However, they differ in other requirements for specific amino acids or peptides as other forms of N. When different forms of carbohydrate are supplied to the rumen, the different groups of micro-organisms will compete for the available N sources (Mathers *et al*, 1981).

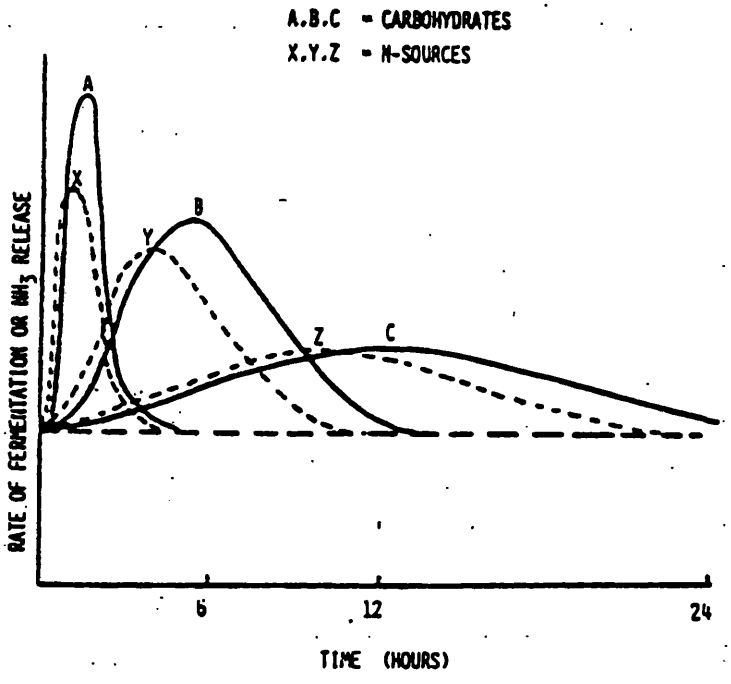

Figure 1.7 Theoretical rumen fermentation rates over time after ingestion of three forms of feed carbohydrate and rumen NH_3 curves required to support protein synthesis from fermentation of these carbohydrates
A = soluble sugars, B = starches and dextrins, C = cell wall carbohydrates
(Johnson, 1976)

1.2.4.3 Rate of Nutrient Release In Silage

Forage proteins are readily soluble and rapidly broken down by the rumen micro-organisms (Ouellet *et al*, 1997). Particularly in grass silage, the protein is broken down so rapidly that NH_3 is produced faster than it can be utilised by the micro-organisms (Satter and Slyter, 1974). This excess NH_3 is absorbed directly from the rumen and excreted in the urine, thus feed protein can be wasted (Thomas and Gill, 1988).

In contrast to the N supply from grass silage, the main source of energy in grass silage is in the form of cell wall contents (hemicellulose and cellulose), which are slowly fermented in the rumen, since most of the soluble carbohydrate are fermented before consumption. A

significant proportion of ME is fermentation end products such as lactic acid and VFA which are a source of energy the ruminant, but cannot be utilised by the rumen micro-organisms to synthesise microbial protein.

1.2.4.4 Rumen Synchrony

Johnson (1976) and Sniffen *et al* (1983) proposed that balancing the rate of supply of energy and N-yielding substrates to rumen micro-organisms can maximise the capture of rumen-degradable N (RDN) and thus optimise microbial growth rate and efficiency. It was therefore suggested that if the capture of RDN was made more efficient, there would be a reduced requirement for DUP, the sources of which are usually expensive, and the excretion of urinary N would be reduced.

To efficiently utilise degraded N and prevent losses by absorbtion, fermentation of OM must supply energy fast enough to meet synthetic abilities of the microbes (Oldham *et al*, 1977). It is the rate of fermentation that can alter protein supply from the rumen and this can be done by changing the energy supply. Therefore, dietary carbohydrate sources are important in determining rumen microbial protein synthesis from NH_3. Oldham *et al* (1977) examined the interaction between dietary carbohydrate and N digestion in sheep by supplying carbohydrate with different rumen fermentation rates. It was concluded that the form of energy supplied did influence duodenal N flow by affecting NH_3-N capture in the rumen, although amino acid content of duodenal N was not really affected.

Herrera-Saldana and Huber (1989) showed that early lactation cows produced more milk when fed a synchronous diet in terms of energy and N supply to the rumen, with barley and

cottonseed meal as rapidly rumen-degradable sources of starch and protein respectively. This was in comparison to a slowly fermented synchronous diet, with milo and brewers' dried grains as the carbohydrate and N sources, and asynchronous diets.

In a second study, Herrera-Saldana *et al* (1990) determined the effect of synchronising starch and protein degradability in the rumen on the nutrient digestibility and microbial protein synthesis by lactating dairy cows. It was concluded that starch degradability affected the utilisation of nutrients in the rumen more than protein degradability and that synchronisation for rapid fermentation with more degradable starch and protein resulted in increased microbial protein passage than unsynchronised or less degradable synchronised diets. No differences were found in DM, OM and CP flow to the small intestine.

The concept of a synchrony index was defined by Sinclair *et al* (1993), and describes the degree of synchrony between hourly supply of energy and N in the rumen which is calculated from the sum of *in situ* degradability data. Sinclair *et al* (1995) examined the effects of synchronising energy and N supply in diets with a similar carbohydrate composition on microbial protein synthesis and rumen fermentation in sheep. This involved the formulation of two diets that were either synchronous or asynchronous for hourly release of energy and N to the rumen. It was found that, although total carbohydrate digested in the rumen was greater for the asynchronous diet compared to the synchronous diet (427g/kg DM compared to 364g/kg DM), (which may have been due to the greater amount of starch in the asynchronous diet, which increased degradability), the efficiency of microbial protein synthesis (g N/kg OM truly degraded in the rumen) was 11-20% greater in animals fed the synchronous diet. From these results, it was concluded that microbial N production was more

efficient when the supply of dietary energy and N were more synchronous.

In contrast to the findings of Sinclair *et al* (1995), Witt *et al* (1996) compared two rates of carbohydrate release in asynchronous and synchronous diets for ram lambs, but found that a synchronous supply of OM and N to the rumen did not increase the efficiency of dietary N capture by the animals. However, in more recent studies by Witt *et al* (1999a, 1999b), when synchronous diets with a rapidly-released source of OM were offered, an improvement in food conversion efficiency was observed.

In a number of studies, the rate of carbohydrate degradation has been shown to be more important than the degree of synchronisation when the effects of synchrony on microbial growth and efficiency are examined. This has been demonstrated both *in vivo* (Herrera-Saldana *et al*, 1990) and *in vitro* (Henning *et al*, 1991). It was later reported by Henning *et al* (1993) that an even rate of carbohydrate supply to the rumen resulted in the greatest flow of microbial protein at the duodenum and the highest efficiency of microbial growth with little effect resulting on the pattern of N supply.

1.2.4.5 Rumen Metabolism & Silage Diets

Herrera-Saldana *et al* (1990) studied supplementing grass silage with concentrates and observed that in dairy cows fed a complete diet with a rapidly-releasing carbohydrate as a supplement, microbial growth and efficiency was greatest.

Synchronising carbohydrate and N source in the rumen in other studies using grass silage as the basal forage has produced variable results. Rooke *et al* (1987) reported that the

continuous intraruminal infusion of sucrose stimulated microbial protein synthesis in cattle fed grass silage, probably due to the improvement in the synchronisation of energy and silage N release. Following this, Rooke and Armstrong (1989) carried out a similar trial, but also infused casein, which was rapidly degraded in the rumen, and the effect was enhanced. However, Khalili and Huhtanen (1991) fed sucrose alongside grass silage and found no increase in microbial protein production.

1.3 EFFECTS OF PATTERN ON NUTRIENT RELEASE ON VFI

1.3.1 INTRODUCTION

For ruminant production to be efficient, then not only must VFI be maximised, but the nutrients supplied to the rumen, and therefore the rumen micro-organisms, must be balanced in terms of degradability of energy and N (Johnson, 1976). This will increase microbial activity within the rumen so that protein supply, and consequently the efficiency of animal production will be improved, as observed by Herrera-Saldana *et al* (1989)

An improvement in microbial activity will improve the degradability of the diet and, therefore the rate of passage through the gastro-intestinal tract will increase (Chamberlain and Wilkinson, 1996). These are important physical factors which inflence VFI, and give a basic explanation of how nutrient release and VFI are inter-related.

1.3.2 EFFECTS OF PATTERN OF NUTRIENT RELEASE ON VFI OF GRASS SILAGE

It is well established that grass silage is highly asynchronous in terms of nutrient release (Satter and Slyter, 1974). This has been shown to decrease microbial efficiency, which may

be an important factor in the control of VFI of grass silage, contributing to its relatively low intake.

Although grass silage has an adequate N content (by chemical analysis), the proteins are readily soluble and rapidly broken down by the rumen micro-organisms, resulting in an inefficient utilisation of the nutrients contained within the forage, as observed by Thomas and Gill (1988) when grass silage was fed as the sole diet for beef cattle. This is mainly due to the rapid release of N in the rumen, which is beyond the capabilities of capture by the rumen micro-organisms. The pattern of release of nutrients is illustrated diagramatically in Figure 1.8, which also demonstrates how the addition of a carbohydrate concentrate can improve the balance between fermentation and protein degradation in a grass silage-based diet (Webster, 1987).

Huhtanen *et al* (1995) found that by supplying a protein supplement to grass silage, the utilisation of grass silage-cereal diets was improved. This was suggested to be due to grass silage being limited in its ability to supply sufficient amino acids and glucose, therefore by feeding supplements that increase the supply of these nutrients, the high potential of these diets could be achieved. It was found, by feeding rapeseed meal within the diets, that total DMI of dairy cows was increased by 0.50kg/day and there was an increase of 0.31kg DMI for each 10g/kg increase in dietary crude protein supplied. The results from these trials follow a similar trend to results reported by Chamberlain *et al* (1989) for grass silage-barley diets. An increase in intake of grass silage by supplying protein supplements was also attributed to improvements in diet digestibility by Oldham (1984). These studies suggest that the metabolic mechanism related to the energy:protein ratio may limit the VFI of grass silage fed alone.

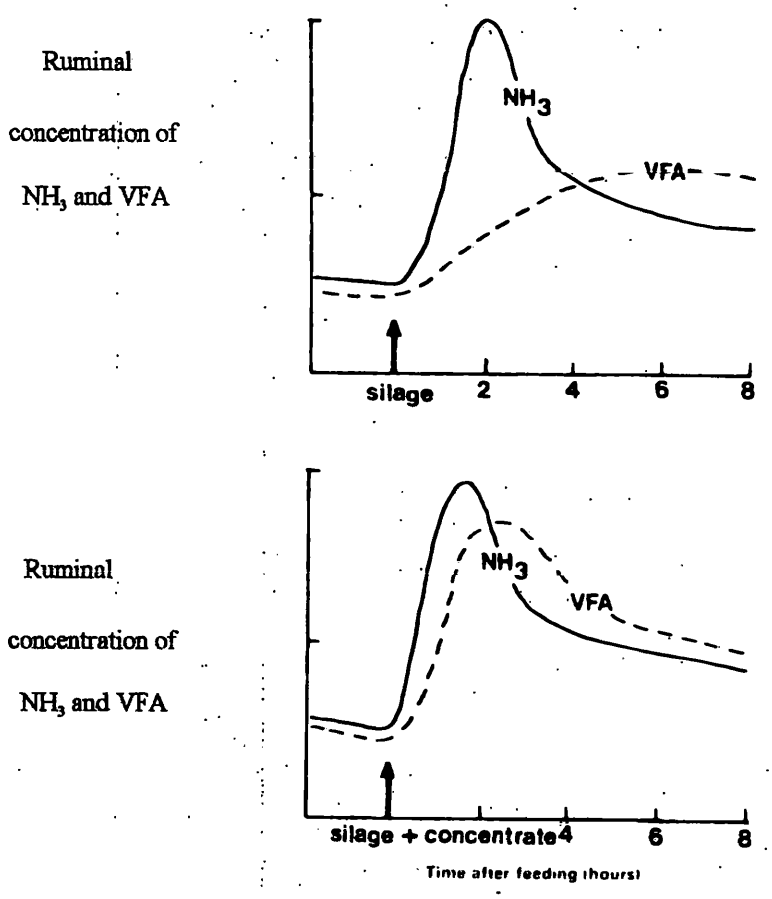

Figure 1.8 Relative rates of production of ammonia (NH_3) and volatile fatty acids in the rumen of dairy cows after feeding (Webster, 1987)

Choung *et al* (1990) abomasally-infused casein and soya protein isolate and observed substantial increases in silage DMI of dairy cows. This was considered to provide evidence for a metabolic control of VFI with the grass silage-barley diets offered when protein supply limits milk production.

A series of trials by Povey (1990), where store lambs were offered grass silage supplemented with varying concentrations of barley and fishmeal resulted in an increase in VFI of the complete diets, resulting in increased daily liveweight gains, suggesting that microbial

efficiency was improved. Supplementing the silage with barley promoted the fastest lamb growth rates (121g/day) and leanest carcasses. The daily liveweight gain increased with increasing fishmeal inclusion, with the maximum gains observed when both barley and fishmeal were included in the supplement.

From these studies, it appears that by manipulating the nutrient supply of grass silage-based diets that the VFI can be improved, therefore the pattern of nutrient release within the rumen, supplied by this forage feed, is considered to be influential on its intake by ruminants.

1.4 GENERAL SUMMARY

VFI is a complex subject which is of primary importance in all animal production systems in terms of diet formulation (McDonald *et al*, 1988). The factors that influence VFI are inter-related and include internal feed-back mechanisms controlled by the animal which, in turn, are influenced by both the physiological status of the animal and the physical characteristics of the feed offered (Forbes, 1995). The VFI of grass silage, in particular, is influenced by a number of factors that specifically apply to the low intake frequently observed when compared to that of other similar feedstuffs (Demarquilly, 1973).

Within the ruminant it is the micro-organisms within the rumen, that live in symbiotic association with the animal, that have a major influence on the control of energy and N metabolism (Webster, 1987). To maximise the efficiency of the ruminant animal, energy and protein metabolism within the rumen must also be maximised by providing these nutrients in the correct proportions (Oldham *et al*, 1977).

Therefore, to achieve an efficient system of ruminant production so that production rates, such as milk yield, daily liveweight gains, feed conversion ratios and reproductive ability are maximised, it is necessary to supply feed that supplies the correct balance of nutrients to the rumen in an amount that relates to the VFI of the animal (Rook and Thomas, 1983).

As grass silage is the most important winter feed for ruminants, it is often incorporated into complete diets or fed as part of a ration (Chamberlain and Wilkinson, 1996). The factors influencing the VFI of this forage must, therefore, be taken into consideration when formulating a diet, whilst simultaneously achieving the required supply of nutrients to the rumen, complicated to some extent by the asynchronous pattern of nutrient release of grass silage (Webster, 1987).

CHAPTER 2

2 DETERMINATION OF THE *IN SITU* DEGRADABILITY CHARACTERISTICS OF NITROGEN, ORGANIC MATTER AND CARBOHYDRATE FRACTIONS IN GRASS SILAGE

2.1 INTRODUCTION

Previous work (Johnson, 1976) has identified that different feeds are degraded at varying rates and to differing degrees within the rumen, depending upon their components, and that within a feedstuff there is a large variability between batches. Therefore, to accurately determine the rate of nutrient release within the rumen, each feed must be characterised prior to incorporation into a diet to ensure that the diet is correctly formulated. Variations in degradability occur due to factors such as growing conditions, storage conditions, physical or chemical treatment and, in the case of silages, efficient ensiling methods (Raymond *et al*, 1986).

All diets for the studies in this research programme were based on one grass silage. Therefore, the objective of the current study was to initially determine the degradability characteristics of the grass silage so that diets differing in their synchrony index could consequently be formulated by the addition of appropriate ingredients. A full chemical analysis was also carried out on the silage.

2.2 MATERIALS AND METHODS

The grass silage that was used was a second cut silage from a short-term ley, consisting primarily of *Lolium perenne*. The grass was cut and wilted for 24 hours then precision chopped (to approximately 5cm) and ensiled on 21st June 1995 with an inoculant and enzyme

additive (Regulator Live; Thomas & Fontaine Limited, Edgton, Craven Arms, Shropshire) added at the rate of 2 litres/tonne of fresh material.

2.2.1 Animals

Four Friesland x Lleyn wether sheep, aged *c.*3 years, weighing *c.*70kg and fitted with permanent rumen cannulae, were housed in individual slatted floor pens and kept under continuous lighting.

2.2.2 Diets

The sheep were fed on a basal diet of the same grass silage as that to be characterised, which was offered *ad libitum* at a rate of 115% of appetite. Feeding was once daily at 9:30am when refusals were also removed and weighed. Water and mineral blocks were available at all times. There was a 10 day adaptation period to the basal diet prior to the incubation of the polysynthetic fibre bags.

2.2.3 Experimental Procedure

A sample of the grass silage was taken from the silage pit and placed in a sealed plastic bag in a refrigerator. The silage was prepared before each incubation period by chopping to approximately 1cm lengths using a homogeniser (Tecator 1094) for 10-12 seconds. This was done to attempt to imitate mastication of the material. Samples of the fresh silage were weighed (approximately 5g DM) into polysynthetic fibre bags of pore size 42μm immediately prior to insertion. Six bags were inserted into the rumen of each animal 30 minutes after feed was offered and retrieved after time periods of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. After retrieval each bag was washed through the cold water cycle of a domestic washing machine

(Hotpoint 95452, programme 7) and dried at 70°C until constant weight. In addition, polysynthetic fibre bags containing a sample of silage were put through the cold water cycle to estimate the rapidly soluble fraction. Sufficient bags were incubated to give a pooled residue of at least 9g dry matter for each animal and time point.

2.2.4 Chemical Analysis

All feed residues were oven-dried and ground through a 1mm screen (Retsch ZM 1000) prior to proximate analysis. Each sample was analysed in duplicate, unless otherwise stated.

2.2.4.a Oven Dry Matter (DM)

A foil tray was heated at 100°C, cooled in a dessicator and weighed. Approximately 250g of silage was accurately weighed into a foil tray and dried at 80°C for 48 hours. The foil tray, containing the dried sample was placed in a dessicator, allowed to cool and weighed. The DM was calculated as:

$$\frac{\text{Weight of residue}}{\text{Weight of fresh sample}} \times 1000 = \text{g/kg of total DM in sample}$$

NB. Oven DM was used throughout these studies. Although this method is suitable for most feedstuffs, toluene DM would have been more appropriate for the calculation of silage DM due to the loss of volatile acids by the oven DM method. Therefore, ME values for the silage are likely to be over-estimated.

2.2.4.b Organic Matter

A crucible was heated at 100°C, cooled in a dessicator and weighed. Approximately 2g of the degradability residue sample or dried silage was accurately weighed into a crucible which was then loaded into a cool muffle furnace. The temperature of the muffle furnace was raised to 450°C for 16 hours. The crucible containing the ash was placed in a dessicator, allowed

to cool and weighed (MAFF, 1986). The content of ash was calculated as:

$$\text{Weight of residue} \times \frac{1000}{2} = \text{g/kg of total ash in sample}$$

Organic matter was calculated as (AOAC, 1980):

$$1000 - \text{g/kg total ash} = \text{g/kg of organic matter in sample}$$

2.2.4.c Nitrogen

The Kjeldahl method of analysis was used where nitrogen in the sample is converted to ammonium-nitrogen by digestion with sulphuric acid (Davidson *et al*, 1970):

Approximately 1g of the dried sample was accurately weighed into an envelope of filter paper (Whatman No. 1) and placed into a digestion tube with two catalyst tablets (Kjeltab CK; Thompson & Cooper Ltd, Runcorn). To this was added 14ml of concentrated sulphuric acid. The tubes containing the samples were placed on a heating block, in a fume cupboard, at 420°C with a Turbosog for 40 minutes until the solution in the flasks turned a turquoise blue colour. The rack was then removed from the heating pad and the flasks allowed to cool for 15 minutes. After cooling, 75ml of water was added to each flask. Ammonium-nitrogen was determined by a Kjeltec Auto Analyser (Tecator model 1035) and automatically calculated back to give a value for percentage nitrogen in the sample. Urea was used as an external standard (MAFF, 1986).

2.2.4.d Fibre

Fibre analysis was carried out by the detergent method (Van Soest *et al*, 1991):

2.2.4.d.i Neutral Detergent Fibre (NDF)

Approximately 0.5g of the sample was accurately weighed into a dried, weighed crucible and placed on the Fibertec apparatus (Tecator; System M 1020 Hot Extractor). Cold neutral

detergent reagent (25ml) (Appendix 2.1) and 0.5ml of octanol was added, brought to the boil and digested for 30 minutes. The heat was turned off, then 25ml of cold neutral detergent reagent and 2ml of alpha-amylase (Fluka; from *Bacilla subtilis*, 55.2U/mg) added (Appendix 2.2). This was brought to the boil and digested for a further 30 minutes. The heat was turned off and the digest filtered and washed three times with 20ml of hot distilled water. After filtration, 25ml of hot distilled water (80°C) and 2ml of alpha-amylase was added. This was allowed to stand for 15 minutes then filtered and washed three times with hot distilled water and once with 20ml of acetone. The crucible was removed from the Fibertec apparatus and dried overnight at 100°C, cooled in a dessicator and weighed. The sample was then ashed for 4 hours at 550°C, cooled and reweighed.

NDF (g) = (crucible + dry fibre weight) - (crucible + ash weight)

$$\text{NDF (\%)} = \frac{\text{NDF weight}}{\text{sample weight}} \times 100$$

2.2.4.d.ii Acid Detergent Fibre (ADF)

Approximately 1g of the sample was accurately weighed into a dried, weighed crucible and placed on the the Fibertec apparatus. Cold acid detergent reagent (100ml) (see Appendix 2.3) was added. This was brought to the boil and allowed to digest for 60 minutes. The heat was turned off and the digest filtered and washed three times with 20ml of hot distilled water and once with 20 ml of acetone. The crucible was removed from the Fibertec apparatus and dried overnight at 100°C. This was cooled in the dessicator and weighed. The sample was ashed for 4 hours at 550°C, cooled and reweighed.

ADF (g) = (crucible + dry fibre weight) - (crucible + ash weight)

$$\text{ADF (\%)} = \frac{\text{ADF weight}}{\text{sample weight}} \times 100$$

2.2.4.d.iii Acid Detergent Lignin (ADL)

The method followed that for ADF, but the crucible was returned to the Fibertec apparatus after drying overnight at 100°C and recording the weight. Following this, 25ml of 72% H_2SO_4 (ADL reagent, Appendix 2.4) was added and extracted cold for 3 hours, mixing every hour. The acid was filtered off and the digest washed three times with 20ml of hot distilled water and once with 20ml of acetone. The crucible was removed from the Fibertec apparatus and dried overnight at 100°C. This was cooled in the dessicator and weighed. The sample was ashed for 4 hours at 550°C, cooled and reweighed.

$$\text{ADL (g)} = (\text{crucible} + \text{dry fibre weight}) - (\text{crucible} + \text{ash weight})$$

$$\text{ADL (\%)} = \frac{\text{ADL weight}}{\text{sample weight}} \times 100$$

2.2.4.e Acid Detergent Insoluble Nitrogen

The method followed that for ADF until the sample had been dried overnight at 100°C and the weight recorded. The dried digest was then analysed for nitrogen using the Kjeldahl method.

2.2.4.f Ether Extract

The oil content of the feed was determined by the Soxtec method (AOAC, 1980):

A Soxtec cup was dried in an oven at 100°C, allowed to cool in a dessicator and weighed. Approximately 2 - 3g of the sample was placed into an extraction thimble and plugged with oil-free cotton wool. The thimble was placed into the cup and placed on the extractor. Petroleum ether (25ml) was pipetted into the extraction cups. The thimble was immersed in the solvent and boiled for 15 minutes. The thimbles were then lifted out of the solvent and rinsed for 10 minutes. The condensed solvent was collected and then evaporated. Finally, the

extraction cups containing the fat were weighed. Fat content was calculated as:

$$\% \text{ fat in sample} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.2.4.g Metabolisable Energy

The Neutral Cellulase Gammanase Digestibility (NCGD) method for the prediction of ME was used for the silage (MAFF, 1985).

The oil was removed from 0.5g of sample by the Soxtec method described previously. The method for NDF was then followed, but after filtering and washing the crucible was removed from the Fibertec apparatus and a dampened subaseal was pushed into the bottom of the crucible. To the residue was added 30ml of buffered cellulase/gammanase (see Appendix 2.5) solution and the crucible capped and shaken. This was incubated at 40°C for 24 hours, shaking again twice to keep the solution mixed. After incubation, the cap and subaseal were removed and the crucible returned to the Fibertec apparatus. The residue was washed three times with 20ml of hot distilled water and once with 20ml of acetone. The crucible was then removed from the Fibertec apparatus and dried overnight at 100°C. This was cooled in the dessicator and weighed. The sample was ashed for 4 hours at 550°C, cooled and reweighed. The indigestible organic matter (IOM) was calculated as a percentage of the dry matter of the original 0.5g of sample.

$$\text{IOM (g)} = (\text{crucible} + \text{dry fibre weight}) - (\text{crucible} + \text{ash weight})$$

$$\text{IOM (\%)} = \frac{\text{IOM weight}}{\text{sample weight}} \times 100$$

$$\text{Ash (g)} = (\text{crucible} + \text{ash}) - (\text{crucible weight})$$

$$\text{Ash (\%)} = \frac{\text{Ash weight}}{\text{sample weight}} \times 100$$

$$\text{NCD (\%)} = 100 - (\text{IOM (\%)} + \text{ash (\%)})$$

$$ME\ (MJ/kg) = 0.14\ NCD + 0.25\ oil$$

2.2.4.h pH of silage

The fresh silage was extracted with water, then the pH of the extract determined (MAFF, 1986):

Approximately 50g of fresh silage was accurately weighed into a 500ml beaker and 125ml of distilled water added. This was left at room temperature for 60 minutes and the extract decanted into a beaker. The pH was recorded using a pH meter (Whatman).

2.2.4.i Ammonium-Nitrogen in silage

Ammonium-nitrogen was extracted with water, made alkaline and the released ammonia removed by distillation and determined by titration:

Approximately 20g of fresh silage was accurately weighed into a 250ml shaking bottle with 100ml of distilled water, capped and shaken on a shaking machine for 60 minutes. The extract was filtered through a No. 1 filter paper into a 100ml conical flask. Ammonium-nitrogen in the extract was determined using the Kjeltec Auto Analyser:

Distilled water (10ml) was pipetted into a 250ml digestion tube and 6.0ml of magnesium oxide (17g ignited oxide/100ml distilled water) suspension added. This was analysed on the Kjeltec Auto Analyser. This method was repeated using a duplicate water blank, the mean titre calculated and the 'Blank' set. The whole procedure was repeated with a standard solution (MAFF, 1986).

$$Factor\ (f) = 0.357 / mean\ titre$$

The procedure was then carried out using the samples.

$$\text{Ammonium-nitrogen (g/kg DM)} = \frac{7 \times T \times f \times (120 - (0.02 \times DM))}{DM \times 10}$$

T = mean titre; DM = g/kg DM in silage

$$\text{Ammonium-nitrogen (\% total N)} = \frac{\text{Ammonium-nitrogen} \times 100}{\text{Total N}}$$

2.2.5 Degradability of Concentrate Ingredients

As rumen degradability is the key component to this series of trials, it was necessary to determine the rate of degradation for each fraction of all the concentrate ingredients to be used during the study. Specific raw ingredients had been incubated for defined lengths of time during a previous study (Witt *et al*, 1999a) and their degradability characteristics consequently determined from the chemical analysis of the dacron bag residues (Appendix 2.6). Using the same method, the degradability data for the DM, N, OM and carbohydrate fractions of the grass silage were calculated from the chemical analysis results obtained. These feeds were stored and available for use in this study.

2.2.6 Calculation Of Degradability Coefficients

The grass silage degradability data were fitted to the first order model of Ørskov and McDonald (1979) for DM, N, OM, hemicellulose (NDF - ADF) and cellulose (ADF - ADL) degradability in the rumen.

$$p = a + b\,(1 - e^{-ct})$$

where: p = amount degraded at time (t)
a = water-soluble fraction
b = potentially degradable fraction
c = rate of degradation
e = natural log
t = time

To determine the presence of possible lag phases for the components of the grass silage, the data were also fitted to a first order model that contained a lag (McDonald, 1981):

$p = a$	up to time t_0
$p = a + b\,(1 - e^{-ct})$	from time t_0 onwards
	where: t_0 = lag phase.

2.2.7 Statistical Analysis

The degradability curves were fitted using Genstat (Lawes Agricultural Trust, 1987).

2.2.8 Synchrony Index for the Rumen Environment

The computer programme SIRE (Synchrony Index for the Rumen Environment) reported by Sinclair *et al* (1993) was used to predict the hourly pattern of nutrient release from the grass silage. Results from the proximate analysis and fibre composition, and degradation characteristics obtained from previous studies were entered into the programme's database. The programme was used to calculate the quantity of each constituent degraded per hour by the initial input of the proportion of each constituent in the diet, dry matter intake (g/day), times of feeding during the day and the outflow rate of solids (k) from the rumen. The programme calculates the hourly nutrient release from the difference between the cumulative amount degraded at successive hours and allocating it to the appropriate hour of the day. For example, the amount of a constituent degraded between the 27th and 28th hour following a meal during the first hour of the day is allocated to the 4th hour of the day. This was carried out so that the hourly quantity of N and OM is achieved and a synchrony index expressed as gN/kgOM could be calculated.

The programme uses the equation:

$$25 - \frac{\sum_{1-24} \sqrt{(25 - \text{hourly N:OM})^2}}{\frac{24}{25}}$$

where: 25 = 25kgN/kgOM truly digested in the rumen. This is assumed to be the optimal ratio (Czerkawski, 1986). Calculations for gN/kgCHO use 32 as the optimal ratio (Sinclair, *et al.* 1991)

From these calculations, if a diet has a synchrony index of 1.0, it supplies N and OM to the rumen at the optimal ratio throughout the 24-hour period. Values of <1.0 indicate the degree of synchrony.

2.3 RESULTS

2.3.1 Grass Silage Analysis

The chemical analysis of the grass silage is presented in Table 2.1. The silage was well-fermented with a pH of 4.08 and NH_3-N present at 0.53g/kg (1.729% of total N). The feed quality was also good with 168.2g/kg CP, 11.5 MJ ME/kg DM and a low DM of 229.7g/kg.

Table 2.1 Grass silage analysis

Component	(g/kg DM)
Dry matter (g/kg)	229.7
Crude protein	168.2
Metabolisable energy (MJ/kg DM)	11.5
Organic matter (g/kg)	899.5
Neutral detergent fibre (g/kg)	476.4
Acid detergent fibre (g/kg)	280.8
Acid detergent lignin (g/kg)	20.7
Acid detergent insoluble N (g/kg)	3.51
Ether extract (g/kg)	37.7
Water-soluble CHO (g/kg)	20.0
pH	4.08
NH_3-N	0.53

2.3.2 Degradability Characteristics

The degradability characteristics of the grass silage are presented in Table 2.2. There was a large soluble N fraction at 0.74, whilst the value for organic matter (OM) was considerably lower at 0.351. However, the rate ('c') of the potentially degradable fraction ('b') was similar for both the N and OM fractions. The rate of degradation of hemicellulose ('c') was greater than that of cellulose, although the potentially degradable fraction ('b') was greater (0.875 and 0.691 for cellulose and hemicellulose, respectively).

Table 2.2 Degradability coefficients of the grass silage characterised

Component	'a'	'b'	'c'	lag	r^2
N	0.740	0.193	0.118	---	90.1
OM	0.351	0.471	0.114	1.864	98.4
Hemicellulose	0.064	0.691	0.103	1.719	88.0
Cellulose	0.000	0.875	0.083	1.076	89.2

2.3.3 Predicted Hourly Degradation

Table 2.3 presents the predicted hourly degradation of the characterised components of the grass silage and shows the asynchronous pattern of nutrient release during rumen degradation over a 24-hour period. When the ratio of gN:kgOM is calculated (Figure 2.1), it can be seen that the grass silage was predicted to supply either an excess of N or OM immediately after feeding, with a deficit of N:OM at times after feeding.

Table 2.3 Predicted hourly degradability (g) of the components of the grass silage characterised using results obtained from the *in situ* degrability work (assuming a DM intake of 1kg totally consumed in hour 1)

Hour	N	OM	gN:kgOM	Cell wall	H.cellulose	Cellulose	gN/kgCHO
1	20.50	316.75	64.71	4.84	12.91	0.78	823.3
2	0.49	6.87	70.89	22.83	3.86	17.19	19.6
3	0.41	40.61	10.13	28.59	11.65	16.24	13.24
4	0.35	34.45	10.09	24.57	10.00	14.14	13.05
5	0.29	29.23	10.05	21.12	8.58	12.32	12.85
6	0.25	24.80	10.01	18.16	7.36	10.73	12.66
7	0.21	21.04	9.98	15.61	6.32	9.34	12.47
8	0.18	17.85	9.94	13.42	5.42	8.13	12.29
9	0.15	15.14	9.90	11.53	4.65	7.08	12.10
10	0.13	12.85	9.86	9.91	3.99	6.17	11.92
11	0.11	10.90	9.83	8.52	3.42	5.37	11.74
12	0.09	9.25	9.79	7.33	2.94	4.68	11.57
13	0.08	7.85	9.75	6.30	2.52	4.07	11.40
14	0.07	6.66	9.71	5.41	2.16	3.55	11.23
15	0.06	5.65	9.68	4.65	1.86	3.09	11.06
16	0.05	4.79	9.64	4.00	1.59	2.69	10.90
17	0.04	4.07	9.60	3.44	1.37	2.34	10.73
18	0.03	3.45	9.57	2.96	1.17	2.04	10.57
19	0.03	2.93	9.53	2.54	1.01	1.78	10.42
20	0.02	2.48	9.50	2.18	0.86	1.55	10.26
21	0.02	2.11	9.46	1.88	0.74	1.35	10.11
22	0.02	1.79	9.42	1.61	0.64	1.17	9.96
23	0.01	1.52	9.39	1.39	0.55	1.02	9.81
24	0.01	1.29	9.35	1.19	0.47	0.89	9.66
Total	23.57	584.29	40.35	223.97	96.03	137.71	90.36

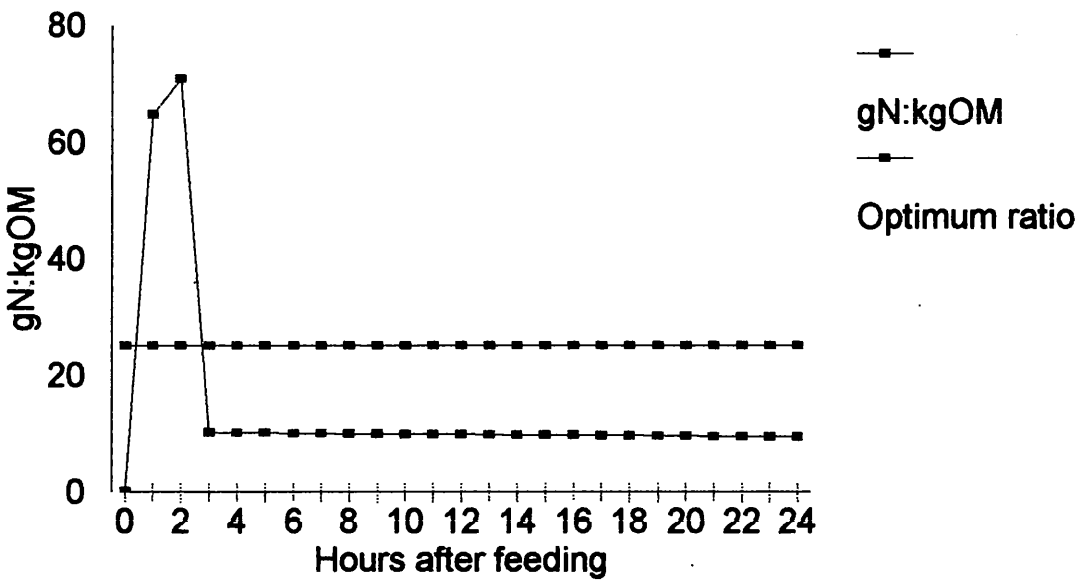

Figure 2.1 Hourly gN/kg OM predicted to be released in the rumen from grass silage when fed once daily

2.4 DISCUSSION

The grass silage characterised during this study was of good quality in terms of nutrient value and fermentation, achieved by efficient production and ensiling methods. These methods are continually being developed to provide improvements in the feed value of grass silage in order to increase productivity and profitability of ruminant livestock enterprises (Flynn, 1988; Thomas and Thomas, 1988).

Degradability characteristics were typical of a grass silage, in that there was a large soluble N fraction relative to that for OM. It is well established that this N escapes capture by rumen micro-organisms, resulting in this N being converted to ammonia in the rumen and absorbed across the rumen wall. This increases blood urea levels (McDonald *et al*, 1988), as the rumen microflora do not possess the necessary energy required for the efficient capture of the N available. For this reason, it is usually necessary to supplement grass silages with a source of protein to make up this shortfall, as well as a starch-based energy source to improve microbial efficiency (Huhtanen *et al*, 1995).

The rate of the potentially degradable fraction was similar for both the N and OM fractions, but the predicted hourly rate of degradation of N:OM illustrates the asynchronous release of N and energy in the rumen with this feed, which has long been established with grass silage (Watson and Nash, 1960). The ratio of gN:kgOM was very high in the first two hours, but after this the ratio fell to approximately 10gN/kgOM. This demonstrates that the optimum ratio was not achieved and why it may be more efficient in terms of animal production to feed the forage with an energy source that closely matches the release of N present in grass silage, and thus improves synchrony. This was illustrated by Herrera-Saldana *et al* (1990) when

silage diets for dairy cows were supplemented with a rapidly-released carbohydrate source.

2.5 CONCLUSION

The degradation pattern of the grass silage characterised was found to be typical of the way in which this type of feed undergoes rumen degradation pattern in terms of the N, OM and carbohydrate fractions. Most N was released in the rumen in the first two hours following feeding, whereas OM and carbohydrate were released more slowly, over a longer time period.

CHAPTER 3

3 INFLUENCE OF THE PATTERN OF NUTRIENT RELEASE FROM GRASS SILAGE AND THE EFFECT OF SUPPLEMENTATION ON THE VOLUNTARY FOOD INTAKE AND METABOLISM OF GROWING LAMBS

3.1 INTRODUCTION

The aim of this study was to determine whether the pattern of nutrient release in grass silage influences the voluntary food intake (VFI) pattern of lambs and whether altering the pattern of nutrient release through supplementation would alter hourly and daily intake.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Eight Lleyn x Charollais wether lambs, weighing c.30kg were housed individually on slatted floors under continuous lighting. Animals were weighed on days 1, 8 and 21 of each period at 1400h.

3.2.2 Diets

The degradability characteristics of the grass silage described in Table 2.1 were entered into the SIRE programme described in Section 2.2. The programme also included the degradability coefficients of feed ingredients that had been determined previously (Witt *et al*, 1999) and presented in Appendix 2.6. Based on these coefficients, assuming a rumen outflow rate of 0.05 and that the 1kg of feed offered per day was consumed in the first hour after feeding, a diet (A) was formulated from ingredients of known degradability to have a similar asynchronous pattern of carbohydrate and nitrogen release in the rumen as the grass silage

(Table 2.3). Diet A was formulated to have a similar content of ME and CP as the grass silage. Similarly, a supplement (S) was formulated to alter the pattern of nutrient release in the rumen and improve the synchrony of energy:nitrogen release in the rumen when fed with diets G and A (Table 3.1).

This resulted in four diets:

G: Grass silage

GS: Grass silage plus supplement (DM 50:50)

A: Diet formulated to have similar pattern of nutrient release to grass silage

AS: Diet A plus supplement (DM 50:50)

Table 3.1 Dietary composition (g/kg DM) for diet A and the synchronous supplement fed to growing lambs

Ingredient	Diet A	Supplement (S)
Wheat straw	261	219
Malt dist dark grains	192	---
Sugar beet pulp	485	---
Barley	---	544
Rapeseed meal	---	237
Urea	20.25	---
Fat (Megalac)	41.75	---

The predicted chemical composition and hourly release of nutrients in the rumen from the four diets is presented in Table 3.2 and 3.3 respectively. All four diets had a similar predicted ME and CP composition, although Diet A had the highest predicted CP content at 176g/kg DM. The two supplemented diets had the highest predicted starch contents at 147 and 148g/kg DM, mainly due to the inclusion of barley, which contributed to their higher total

carbohydrate values. The grass silage had a considerably lower DUP than the other diets at 18g/kg DM. The carbohydrate fraction of the two supplemented diets were more degradable than those of the unsupplemented diets (339 and 334g/kg DM v 246 and 234g/kg DM for grass silage + supplement, Diet A + supplement, grass silage and Diet A, respectively). The cell wall components of Diet A were much less degradable than other diets offered at 208g/kg DM, whilst the cellulose component of the grass silage was most degradable at 137g/kg DM. The RDP content was greatest for the grass silage, at 147 compared with Diet A at 128g/kg DM, whilst the DUP content in Diet A was almost double that of grass silage (47 and 21 g/kg DM, respectively).

The synchrony indices calculated by the SIRE programme show that the grass silage was the most asynchronous diet, both in terms of gN/kgOM and gN/kgCHO. The two supplemented diets show relative synchrony in terms of nutrient release in the rumen.

Table 3.3 shows the predicted hourly degradability of Diet A and illustrates that the pattern of nutrient release and synchrony indices for gN/kgOM and gN/kgCHO are similar to the grass silage throughout the day on an hourly basis.

Table 3.2 Predicted chemical composition (g/kg DM), degradability and synchrony index of grass silage, asynchronous diet (A), grass silage + supplement and diet A + supplement (50/50 on a DM basis) fed to growing lambs

Component (g/kg DM)	Grass silage (G)	Diet A (A)	Grass silage + supplement (GS)	Diet A + supplement (AS)
ME (MJ/kg DM)	11.5	11.4	11.5	11.5
Crude protein	168	176	165	169
Total carbohydrate	496	556	624	654
Starch	0	3	147	148
Water-soluble CHO	20	45	37	36
Cell wall	476	544	440	475
Hemicellulose	195	251	196	224
Cellulose	260	238	199	188
Ether extract	38	58	27	38
DUP	18	34	28	36
Effective degradability coefficients (g/kg): - assuming an outflow rate of 0.05				
Organic matter	0.584	0.426	0.582	0.503
Carbohydrate	0.246	0.234	0.339	0.334
Nitrogen	0.024	0.021	0.020	0.019
Cell wall	0.224	0.208	0.172	0.163
Hemicellulose	0.096	0.124	0.087	0.101
Cellulose	0.137	0.095	0.091	0.069
Daily ratio total gN/kgOM	40	48	35	37
Daily ratio total gN/kgCHO	96	88	59	56
RDP (g/kg DM)	147	128	126	117
UDP (g/kg DM)	21	47	38	52
Synchrony index:				
OM	0.30	0.51	0.85	0.88
CHO	-0.61	0.60	0.84	0.75

NB. See Appendix 2.6 for measured 'a', 'b' and 'c' values

Table 3.3 Predicted hourly degradability (g) of the components of diet A using results obtained from the *in situ* degrability work (assuming a DM intake of 1kg totally consumed in hour 1)

Hour	N	OM	gN:kgOM	Cell wall	H.cellulose	Cellulose	gN/kgCHO
1	16.09	203.32	79.14	61.55	51.27	17.61	762.37
2	0.50	21.86	22.85	14.30	10.47	6.46	121.05
3	0.39	19.56	19.81	14.32	8.98	5.77	98.43
4	0.31	17.56	17.90	12.91	7.71	5.15	89.29
5	0.26	15.78	16.73	11.65	6.63	4.60	83.93
6	0.23	15.96	14.28	10.51	5.70	4.11	46.31
7	0.20	15.08	13.31	9.48	4.91	3.67	37.12
8	0.18	13.65	13.12	8.56	4.24	3.84	36.49
9	0.16	12.35	13.05	7.73	3.66	5.55	36.19
10	0.15	11.18	13.05	6.98	3.17	4.96	36.10
11	0.13	10.13	13.11	6.31	2.74	4.43	36.15
12	0.12	9.17	13.20	5.70	2.38	3.95	36.29
13	0.11	8.31	13.31	5.15	2.07	3.53	36.50
14	0.10	7.53	13.44	4.66	1.80	3.15	36.74
15	0.09	6.82	13.58	4.22	1.57	2.82	37.02
16	0.09	6.18	13.74	3.82	1.37	2.52	37.32
17	0.08	5.61	13.90	3.45	1.19	2.25	37.64
18	0.07	5.08	14.07	3.13	1.04	2.01	37.97
19	0.06	4.61	14.25	2.83	0.92	1.80	38.32
20	0.06	4.18	14.43	2.57	0.80	1.61	38.67
21	0.05	3.79	14.62	2.33	0.71	1.44	39.04
22	0.05	3.44	14.82	2.11	0.62	1.29	39.42
23	0.05	3.12	15.02	1.91	0.55	1.15	39.81
24	0.04	2.83	15.23	1.74	0.48	1.03	40.20
Total	19.59	427.10	45.86	207.90	124.95	94.67	219.27

The concentrate feed ingredients from which Diet A and the supplement (S) were made were prepared by grinding through a mill (sieve size 2mm) before being accurately weighed out and thoroughly mixed in a Gardner mixer.

Each 25kg batch of concentrate feed was sub-sampled and the grass silage sampled weekly. All samples were frozen prior to analysis.

Animals were offered their allocated diet *ad libitum* at a rate of 115% of appetite. Refusals were collected daily and intake requirements calculated for the following day. Feeding was once daily at 9:30am. Water and mineral blocks were available at all times.

3.2.3 Experimental Procedure

A replicated 4 x 4 Latin square design was used, where two groups of four animals were allocated at random to four dietary treatments in four periods each of 32 days duration. During each period there were 14 days adaptation followed by an 18 day sampling period. On day 14 of each period the animals were fitted with faecal harnesses. Faecal collection was carried out by lining the pouch of the harness bag with a plastic bag which was emptied and replaced daily at 1000h. The faeces were weighed and a 10% subsample frozen at -20°C prior to analysis. Faeces were collected for a 7-day period. During this period, daily intake (kg/day) was recorded. Samples of feed refusals were taken daily and frozen for subsequent analysis.

On day 21 an electronic balance (Model 3359, Avery Limited) was placed underneath each food trough. These were connected to a central data logger (Grants 1250 Series Squirrel

Meter) which recorded the voltage from each balance at 5 minute intervals. This voltage was calibrated for weight for each balance. A closed circuit time lapse video (Panasonic AG-6024B) and low light intensity camera system was also set up to record a visual assessment of intake pattern by a time-lapse method, as a back-up for data logger readings. Voluntary food intake pattern was recorded for three consecutive days. The first 24-hour period was used for analysis unless the daily intake (kg DM) was more than 5% different from the mean intake for that animal (as recorded during the faecal collection period). If the daily intake differed by more than 5%, then a subsequent day's sample was used for analysis. On collecting the data from the logger, the hourly intake of each animal was calculated by the addition of twelve 5-minute readings starting at the time of feeding. If an animal was eating at the point of the hourly reading, the intake was carried forward to the following time interval.

Blood sampling was carried out at 7 time-points on days 26 (0830h and 2030h), 27 (0430h and 1630h) and 28 (1230h, 1030h and 0030h). Two 7ml samples were taken by jugular puncture at each time-point into potassium oxalate and lithium heparin vacutainer tubes The samples were centrifuged at 1500G for 15 minutes (MSE Contaur 30 centrifuge) and the plasma frozen at -30°C prior to analysis.

Rumen sampling was carried out at 5 time-points on days 29 (0830h, 1230h and 2030h) and 30 (1030h and 1630h). This was carried out by oesophageal tube using a hand-operated vacuum pump. The first 20ml of extracted fluid was discarded to eliminate contamination by saliva. On extraction, the fresh rumen fluid was strained through muslin. The pH was taken immediately, using a glass amplified electrode (HI 1290) pH meter (Hanna Instruments;

Piccolo 2) and the rumen fluid acidified to less than pH2, using concentrated HCl, and frozen.

The experimental procedure followed in each period is shown in Table 3.4.

Table 3.4 Experimental procedure for growing lambs fed four diets differing in their pattern of nutrient release in the rumen

Day	Procedure
1	Animals weighed
1 - 4	Diet changeover period
1 - 14	Adaptation period
8	Animals weighed
14	Attach faecal harnesses
15 - 21	Faecal collection
21	Set up data logger and video system
	Animals weighed
22 - 25	Record voluntary food intake pattern
26 - 28	Blood sampling
29 - 30	Rumen sampling

3.2.4 Chemical Analysis

3.2.4.a Proximate Analysis of Feed and Faecal Samples

All feed and faecal samples were oven-dried and ground through a 1mm screen (Retsch ZM 1000) prior to proximate analysis. Each sample was analysed in duplicate for DM, OM, N and NDF as described in Section 2.2.4. Additionally, feed samples were analysed for ADF, ADL and ether extract.

3.2.4.b Blood Analysis: Urea and Beta-hydroxybutyrate (BHB)

The serum samples were thawed at room temperature and transferred to labelled vials. A Bayer Technicon RA-1000 auto-analyser was used in conjunction with the appropriate chemical kit for each metabolite: Urea - Bayer Diagnostics (catalogue no. T11-1822)

BHB - Sigma Diagnostics (catalogue no. 310-UV)

3.2.5 Statistical Analysis

Results were evaluated by analysis of variance (ANOVA) using the statistical package Genstat 5 (Lawes Agricultural Trust, 1987). The error degrees of freedom were subdivided into three:

Effect of synchronising nutrient release:	G and A v GS and AS	(C)
Effect of presence of fermentation products:	G and GS v A and AS	(S)
Presence of an interaction		(I)

3.3 RESULTS

3.3.1 Daily Intake And Whole Tract Digestibility

Results for the mean values of DMI, NDF, OM and N digestibility are presented in Table 2.8. Daily DMI was greatest for diets A and AS, and lowest for the grass silage, with the supplemented grass silage having an intermediate intake. The presence of grass silage had the most significant effect ($p<0.001$) on DMI. The effect of supplementing silage to improve hourly synchrony of N and energy release in the rumen appeared to be significant ($p<0.01$), but the interaction ($p<0.01$) indicates that this was only applicable for the grass silage.

The DM digestibility was greatest for the grass silage diets and showed an inverse trend to

those for DMI. There was a significant difference ($p<0.001$) between the grass silage-based and diets A and AS, but supplementing either the grass silage or diet A had no significant effect ($p \geq 0.05$) on DM digestibility.

Table 3.5 Daily DMI (kg/day) and whole tract digestibility coefficients of DM, NDF, OM and N

	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
DMI	0.937	1.322	1.581	1.574	0.0859	***	**	**
DM dig	0.7639	0.7153	0.612	0.6325	0.01222	***	NS	***
NDF dig	0.7645	0.6303	0.5906	0.5164	0.01779	***	***	*
OM dig	0.7689	0.7155	0.6299	0.6444	0.01260	***	*	**
N dig	0.7946	0.7521	0.7019	0.6359	0.02290	***	**	NS

S = presence of silage ie. effect of fermentation products
C = presence of concentrate supplement ie.effect of synchronising hourly supply of N and E to the rumen
I = effect of any interaction

NDF digestibility was significantly higher ($p<0.001$) for the grass silage diets. Synchronising the release of nutrients in the rumen appeared to significantly reduce ($p<0.001$) the fibre digestibility, with a slight interaction effect present ($p<0.05$), due to the greater reduction in digestibility for the supplementing of the grass silage.

The digestibility of OM followed a similar trend to that of NDF, with the grass silage diets being significantly ($p<0.001$) more digestible than diets A and AS. The effect of supplementing either the grass silage or diet A was significant ($p<0.05$), but the presence of

an interaction ($p<0.01$) indicates that this was due to the greater reduction of OM digestibility when supplementing the grass silage.

N digestibility was significantly greater ($p<0.001$) when the animals were fed grass silage diets than the diets A and AS. A significant reduction ($p<0.01$) in digestibility was also seen by the addition of the supplement. No significant interaction ($p\geq0.05$) was present.

3.3.2 Intake Pattern

Table 3.6 shows the hourly DMI of the four diets. The first hour after feeding shows that DMI was greatest during this period. Most feeding activity occured during daylight hours, although there was some feeding activity during the night and this increased in the early morning. Intake values for diets A and AS were significantly higher ($p<0.01$) than those of the grass silage diets during the first hour after feeding. However, in the second hour, the diets GS and AS had significantly greater ($p<0.001$) intakes than diets G and A, indicating that the animals continued consuming the more synchronous diets, but the intake of the less synchronous diets was reduced following the initial meal.

The following hours showed no significant differences ($p\geq0.05$) between diets until hour 11, when the intake of the diets A and AS was significantly greater ($p<0.05$) than those of the grass silage-based diets.

There were no significant differences ($p\geq0.05$) in DMI between the four diets until hour 23, when animals consumed a significantly greater ($p<0.05$) amount of diets G and A than that of diets GS and AS. This trend continued into hour 24, where there was also a significant

difference ($p<0.05$) between the grass silage-based diets and diets A and AS. However, this is likely to be due to the increased intake of diet AS during hour 24.

The hourly pattern of DMI is presented in Figure 3.1, illustrating the similar hourly intake patterns of the four diets.

Table 3.6 Hourly food intake pattern (kg DM)

Hour	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
1	0.143	0.279	0.372	0.398	0.0833	**	NS	NS
2	0.054	0.115	0.071	0.198	0.0340	NS	***	NS
3	0.049	0.102	0.077	0.084	0.0319	NS	NS	NS
4	0.054	0.073	0.097	0.093	0.0337	NS	NS	NS
5	0.0229	0.0503	0.0462	0.0336	0.02377	NS	NS	NS
6	0.077	0.110	0.140	0.128	0.0328	NS	NS	NS
7	0.050	0.046	0.104	0.055	0.0388	NS	NS	NS
8	0.065	0.119	0.117	0.097	0.0370	NS	NS	NS
9	0.059	0.082	0.139	0.111	0.0299	NS	NS	NS
10	0.086	0.099	0.086	0.084	0.0346	NS	NS	NS
11	0.075	0.097	0.144	0.168	0.0296	*	NS	NS
12	0.051	0.101	0.134	0.078	0.0485	NS	NS	NS
13	0.0430	0.0546	0.0509	0.0444	0.02110	NS	NS	NS
14	0.0080	0.0247	0.0221	0.0304	0.01331	NS	NS	NS
15	0.0291	0.0476	0.0351	0.0244	0.01386	NS	NS	NS
16	0.0360	0.0454	0.0285	0.0243	0.02042	NS	NS	NS
17	0.0325	0.0133	0.0357	0.0178	0.01930	NS	NS	NS
18	0.0354	0.0332	0.0353	0.0266	0.01662	NS	NS	NS
19	0.0120	0.0161	0.0110	0.0242	0.01087	NS	NS	NS
20	0.0057	0.0086	0.0090	0.0067	0.00795	NS	NS	NS
21	0.0252	0.0228	0.0333	0.0532	0.01996	NS	NS	NS
22	0.0133	0.0492	0.0353	0.0533	0.02260	NS	NS	NS
23	0.0228	0.0076	0.0661	0.0220	0.01938	NS	*	NS
24	0.020	0.040	0.031	0.130	0.0338	*	*	NS

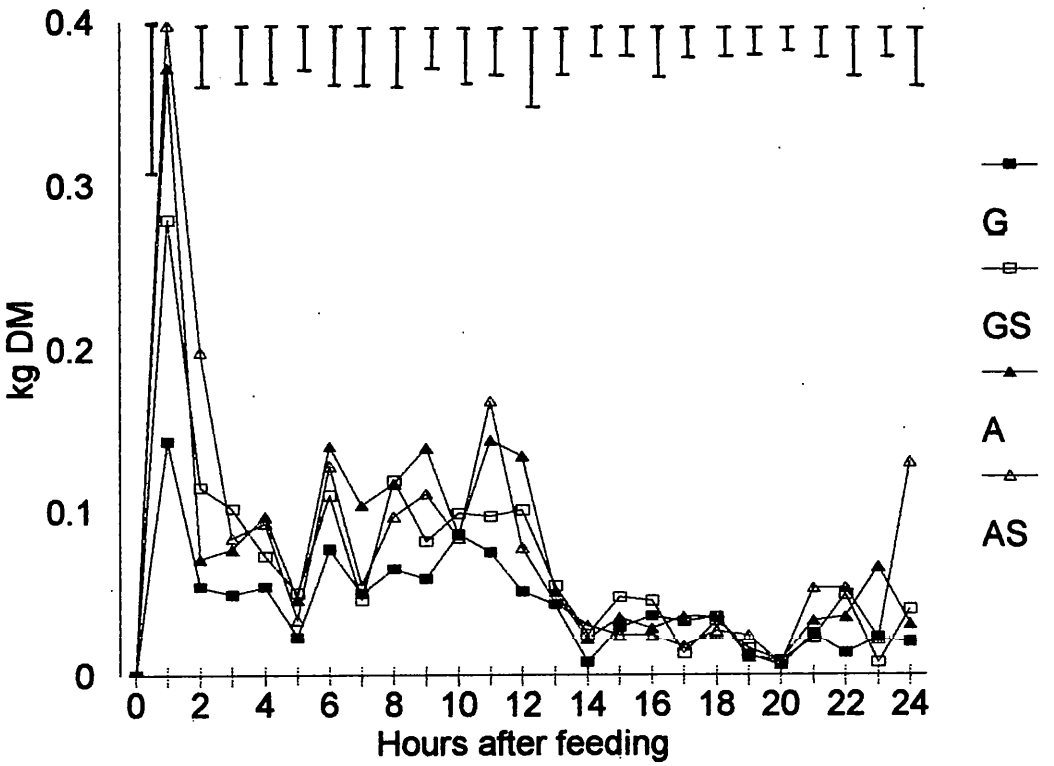

Figure 3.1 Hourly food intake pattern (kg DM) of growing wether lambs offered diets *ad libitum*

When hourly DMI is presented as a proportion of total daily DMI, the pattern of intake appears similar throughout the day with few significant differences seen in Table 3.7. However, during hour 2, there was a significantly higher ($p<0.01$) consumption of the synchronous diets compared to the asynchronous diets.

Table 3.7 Hourly food intake (g DM as % of total daily DM intake)

Hour	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
1	13.3	17.0	19.0	20.1	3.79	NS	NS	NS
2	4.92	6.47	3.61	9.69	1.795	NS	**	NS
3	4.67	6.31	4.03	4.70	1.872	NS	NS	NS
4	5.17	4.35	4.93	4.55	1.877	NS	NS	NS
5	2.21	3.04	2.38	1.67	1.562	NS	NS	NS
6	6.87	6.48	7.12	6.31	1.748	NS	NS	NS
7	4.95	3.12	5.52	2.82	2.296	NS	NS	NS
8	6.40	7.40	6.07	4.79	1.898	NS	NS	NS
9	5.47	5.02	7.22	5.97	1.941	NS	NS	NS
10	7.79	6.36	4.59	4.34	1.935	NS	NS	NS
11	6.77	6.07	7.38	8.57	1.716	NS	NS	NS
12	4.57	5.77	7.19	4.13	2.721	NS	NS	NS
13	3.93	3.09	2.67	2.05	1.309	NS	NS	NS
14	0.83	1.57	1.12	1.50	0.844	NS	NS	NS
15	2.94	3.13	1.90	1.24	1.295	NS	NS	NS
16	3.74	2.74	1.43	1.19	1.375	NS	NS	NS
17	2.89	0.87	1.98	0.85	1.299	NS	NS	NS
18	2.93	2.20	1.81	1.22	1.096	NS	NS	NS
19	1.41	0.93	0.62	1.21	0.751	NS	NS	NS
20	0.64	0.63	0.48	0.33	0.584	NS	NS	NS
21	2.30	1.43	1.83	2.95	1.393	NS	NS	NS
22	1.15	2.88	1.88	2.51	1.196	NS	NS	NS
23	2.28	0.56	3.53	1.04	1.086	NS	*	NS
24	1.84	2.52	1.67	6.32	1.801	NS	NS	NS

The proportions of grass silage diets consumed were greater than those of diets A and AS during hours 10 and 16, but these values were not significantly different ($p \geq 0.05$). Variation between diets appeared to increase after hour 5, although statistical analysis shows that this was not significant ($p \geq 0.05$).

The proportion of daily intake of asynchronous diets (G and A) consumed was significantly greater ($p < 0.05$) during hour 23 and the values are close to being significant during hour 24. However, animals offered the grass silage appeared to have a more sustained hourly intake, especially during the later hours, than other diets.

The results of hourly DMI on an accumulative basis are presented in Table 3.8. During hour 1, the intake of the grass silage diets was significantly lower ($p < 0.01$) than those based on diet A (A and AS), but there was no effect of synchronising either silage. However, from hour 2 onwards, there was a significant increase ($p < 0.05$) in intake when the diets were synchronised. This had an effect on both silage-based diets until hour 10, when the effect of improving hourly synchrony only improved intake significantly in the grass silage diet.

Table 3.8 Hourly accumulative food intake pattern (kg DM)

Hour	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
1	0.143	0.279	0.372	0.398	0.0833	**	NS	NS
2	0.197	0.394	0.443	0.597	0.1022	**	*	NS
3	0.247	0.497	0.520	0.681	0.1087	**	*	NS
4	0.301	0.569	0.617	0.773	0.1154	**	*	NS
5	0.324	0.620	0.664	0.807	0.1181	**	*	NS
6	0.401	0.730	0.804	0.935	0.1324	**	*	NS
7	0.451	0.776	0.907	0.990	0.1205	***	*	NS
8	0.516	0.894	1.025	1.087	0.1288	**	*	NS
9	0.576	0.976	1.163	1.198	0.1248	***	*	NS
10	0.662	1.075	1.249	1.282	0.1210	***	*	*
11	0.737	1.172	1.393	1.450	0.1184	***	**	*
12	0.788	1.273	1.526	1.528	0.1205	***	*	*
13	0.831	1.327	1.577	1.573	0.1181	***	**	**
14	0.839	1.352	1.599	1.603	0.1121	***	**	**
15	0.868	1.400	1.634	1.627	0.1122	***	**	**
16	0.904	1.445	1.663	1.652	0.1157	***	**	**
17	0.937	1.458	1.699	1.669	0.1032	***	**	**
18	0.972	1.491	1.734	1.696	0.1075	***	**	**
19	0.984	1.508	1.745	1.720	0.1074	***	**	**
20	0.990	1.516	1.754	1.727	0.1057	***	**	**
21	1.015	1.539	1.787	1.780	0.1035	***	**	**
22	1.028	1.588	1.823	1.833	0.1047	***	**	**
23	1.051	1.596	1.889	1.853	0.0999	***	**	***
24	1.072	1.636	1.920	1.984	0.0865	***	***	***

Blocking intake into periods of greater than 1 hour gave slightly different results to examining DMI on an hourly basis. Table 3.9 shows the results of the hourly DMI being blocked into 4, 6, 8 and 12 hour sections. Animals fed the grass silage diets had a significantly lower ($p<0.05$) intake than those fed diets A or AS during the first three 4-hourly blocks, although the differences in the synchronous diets were not significant ($p\geq0.05$) for the second and third blocks. There was also a significant increase ($p<0.05$) in intake of the synchronous diets in the first four hours in comparison to the asynchronous diets. This is seen again in the last four hours of the day.

Blocking hourly DMI into 6-hourly sections showed similar results, with animals offered grass silage diets having a significantly lower ($p<0.01$) DMI during the first, second and final block. However, the intake of the supplemented grass silage was not significantly lower ($p\geq0.05$) than that of diet AS, as can be seen by the presence of an interaction ($p<0.01$). Supplementing both diets G and A significantly increased ($p<0.05$) DMI during the first and last 6-hour block.

The first 8-hourly DMI was significantly greater ($p<0.05$) in animals offered the synchronous diets (GS and AS), however, the difference between the diets A and AS was relatively small.

When the daily intake was split into two 12-hourly blocks, animals offered the silage diets (G and GS) had significantly lower ($p<0.001$) intakes than those offered diets A and AS. Synchronisation resulted in a significantly greater ($p<0.05$) intake during the first 12 hours, but this only applied to the grass silage.

Table 3.9 Blocked food intake pattern (kg DM)

Hours	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
1 - 4	0.301	0.569	0.617	0.773	0.1154	**	*	NS
5 - 8	0.215	0.325	0.407	0.314	0.0498	*	NS	**
9 - 12	0.272	0.378	0.502	0.441	0.0451	***	NS	*
13 - 16	0.116	0.172	0.137	0.123	0.0344	NS	NS	NS
17 - 20	0.0855	0.0713	0.0909	0.0753	0.02328	NS	NS	NS
21 - 24	0.082	0.120	0.166	0.257	0.0427	**	*	NS
1 - 6	0.401	0.730	0.804	0.935	0.1324	**	*	NS
7 - 12	0.387	0.542	0.723	0.593	0.0571	***	NS	**
13 - 18	0.184	0.219	0.207	0.168	0.0435	NS	NS	NS
19 - 24	0.099	0.144	0.186	0.288	0.0421	**	*	NS
1 - 8	0.516	0.894	1.025	1.087	0.1288	**	*	NS
9 - 16	0.388	0.551	0.638	0.565	0.0467	***	NS	**
17 - 24	0.167	0.191	0.257	0.332	0.0543	**	NS	NS
1 - 12	0.788	1.273	1.526	1.528	0.1205	***	*	*
13 - 24	0.283	0.363	0.393	0.456	0.0632	*	NS	NS

Blocking the proportions of hourly DMI from Table 3.7 showed that the differences between diets were not significantly different ($p \geq 0.05$) in most instances. However, the results presented in Table 3.10 show that the general trend when blocking for 4, 6 and 8-hourly proportion consumed resulted in animals offered the synchronous diets (GS and AS) having a greater intake in the first block, as well as for the supplemented grass silage (GS) when the

daily DMI proportion was split into 12 hours.

During the rest of the day, the asynchronous diets (G and A) were eaten in greater proportions than the synchronous diets. However, diet AS was consumed in a greater proportion than diet A in the second 12-hour block.

Table 3.10 Blocked food intake (g DM as % of total daily DM intake)

	Treatment Means					Significance of main effects		
Hours	G	GS	A	AS	s.e.d.	S	C	I
1 - 4	28.1	34.2	31.6	39.0	4.84	NS	NS	NS
5 - 8	20.43	20.07	21.08	15.59	2.583	NS	NS	NS
9 - 12	24.6	23.2	26.4	23.0	3.09	NS	NS	NS
13 - 16	11.44	10.53	7.12	5.98	2.189	*	NS	NS
17 - 20	7.87	4.62	4.89	3.60	1.471	NS	*	NS
21 - 24	7.58	7.38	8.91	12.82	2.748	NS	NS	NS
1 - 6	37.1	43.7	41.1	47.0	5.47	NS	NS	NS
7 - 12	36.0	33.8	38.0	30.6	3.55	NS	NS	NS
13 - 18	17.26	13.59	10.91	8.04	2.601	**	NS	NS
19 - 24	9.63	8.93	10.01	14.36	2.747	NS	NS	NS
1 - 8	48.5	54.2	52.7	54.6	4.94	NS	NS	NS
9 - 16	36.1	33.8	33.5	29.0	3.23	NS	NS	NS
17 - 24	15.4	12.0	13.8	16.4	3.29	NS	NS	NS
1 - 12	73.1	77.5	79.1	77.6	3.81	NS	NS	NS
13 - 24	26.9	22.5	20.9	22.4	3.81	NS	NS	NS

3.3.3 Blood Metabolites

Figures 3.2 and 3.3 present the results of blood plasma β-hydroxybutyrate (BHB) and urea, respectively, from samples throughout the day.

At 1 hour after feeding, there was no significant difference ($p \geq 0.05$) in BHB concentrations between animals offered any of the dietary treatments. When sampled 3 hours after feeding, animals fed the grass silage-based diets (G and GS) had significantly higher ($p<0.05$) concentrations of plasma (BHB) than those fed the diets A and AS ($p<0.05$), whilst plasma BHB levels were greater for both synchronised diets (GS and AS) than the asynchronous diets (G and A) ($p<0.01$). Improving the synchrony of hourly nutrient supply of nutrients resulted in significantly elevated ($p<0.05$) plasma BHB levels throughout the day, except at one hour before and one hour after feeding. In contrast to the significantly greater ($p<0.05$) plasma BHB concentrations at hour 3 for the grass silage-based diets, at hour 19 values were significantly lower ($p<0.05$) than those of the diets A and AS.

Figure 3.2 illustrates plasma BHB concentrations, with animals offered diets GS and AS having greater levels through the day. The grass silage diets appeared to follow similar trends, although the synchronised diets had continually higher levels. Animals fed diet A remained at a relatively low level compared to other diets, with little fluctuation.

Plasma urea levels were significantly lower ($p<0.001$) throughout the day in lambs offered diets based on grass silage (G and GS) than those offered diets based on diet A (A and AS). There was an interaction ($p<0.05$) between basal diet and supplementation, with animals offered diet AS having lower plasma urea levels throughout the day than those offered diet

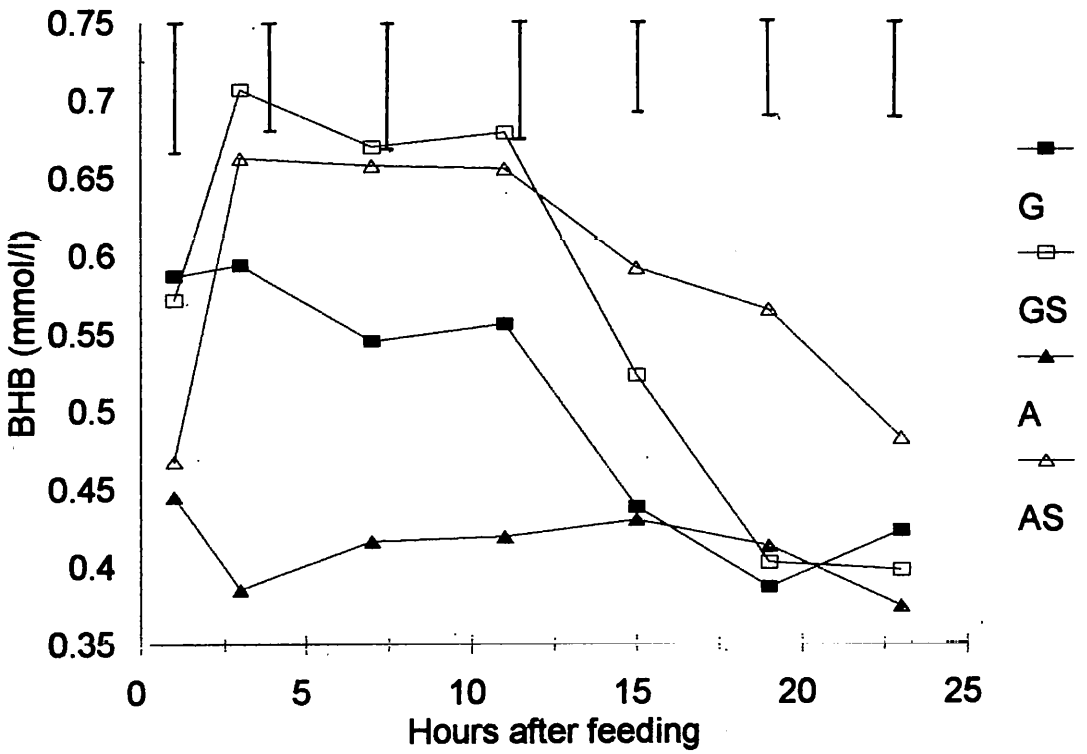

Figure 3.2 Blood plasma BHB concentrations of growing wether lambs offered diets *ad libitum*

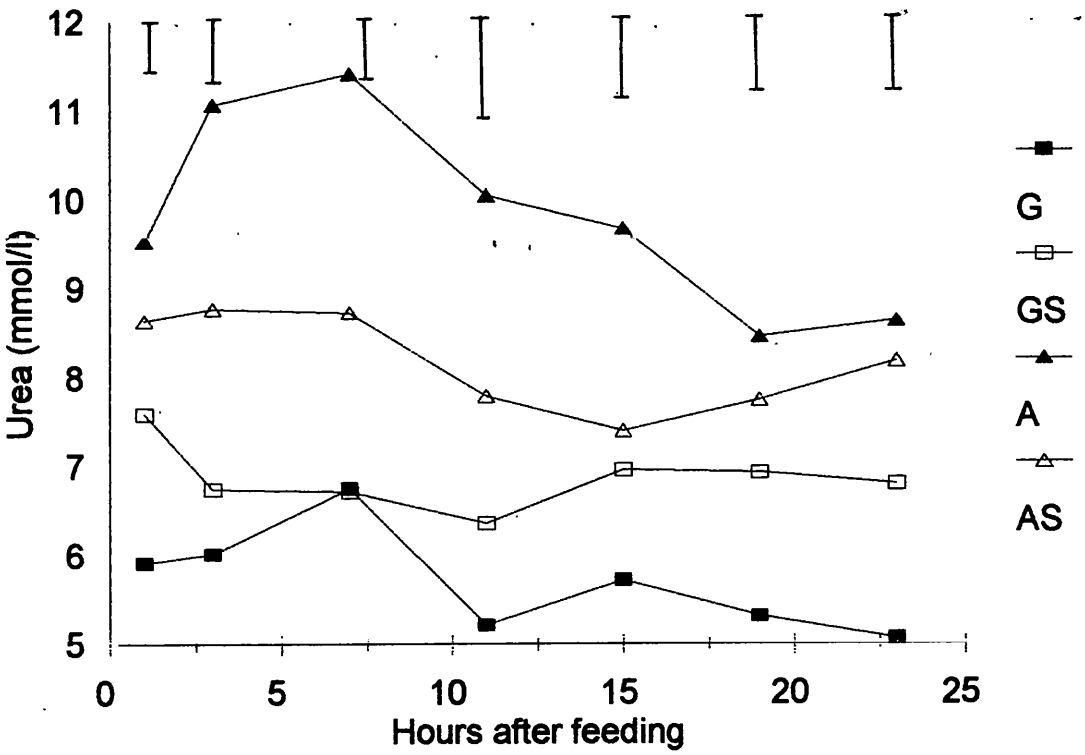

Figure 3.3 Blood plasma urea concentrations of growing wether lambs offered diets *ad libitum*

A, whilst those offered diet GS had higher levels throughout the day than those offered diet G. The highest plasma urea concentrations were recorded in animals offered diet A, however the effect of supplementing either diet G or diet A was not significant ($p \geq 0.05$), except at hour 7.

Figure 3.3 illustrates plasma urea concentrations, with animals offered diet A having the greatest levels of plasma urea through the day. The grass silage diets remained at a relatively low level compared to the diets based on diet A. Animals offered diet AS had intermediate levels of plasma urea, remaining lower than diet A, but higher than diets G and GS throughout the day.

3.3.4 Rumen pH

The pH measurements of rumen fluid samples taken through the day are presented in Figure 3.4. The results show that the rumen pH of animals offered the grass silage remained higher than other those offered diets GS, A and AS throughout the day.

The pH within the rumen of animals fed the grass silage diets was significantly higher ($p<0.05$) throughout the day than those fed diets A and AS. However, the presence of an interaction ($p<0.05$) at hours 3, 12 and 23 post-feeding indicated that the difference between the more synchronous diets is smaller. Synchronising the diet significantly lowered ($p<0.05$) rumen pH, except at 8 hours after feeding.

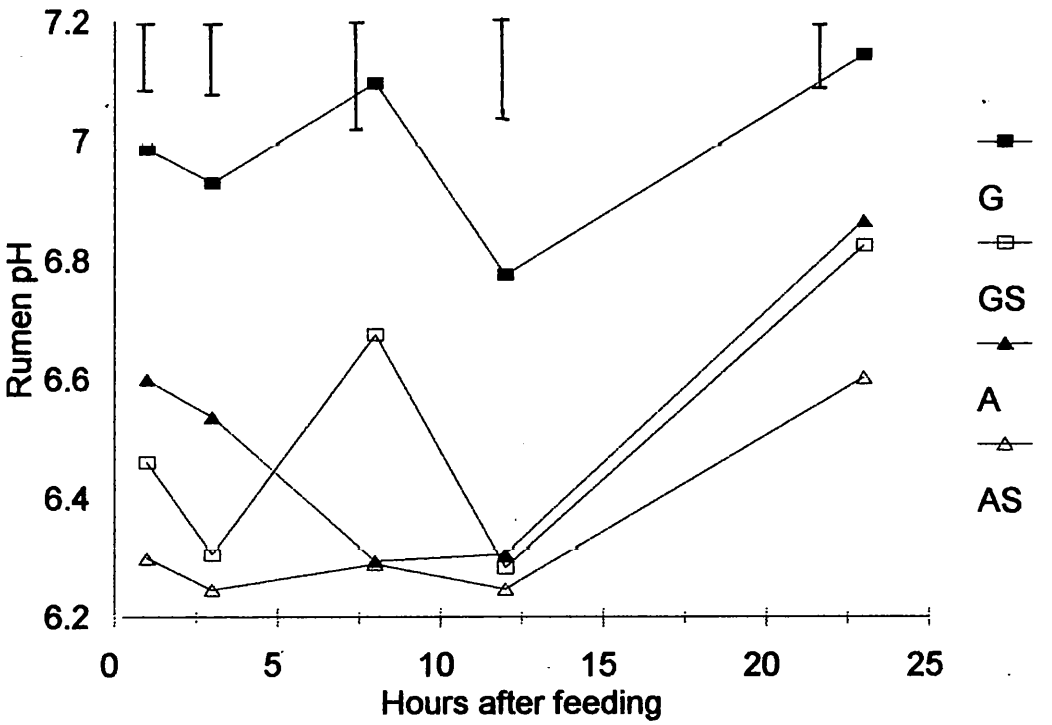

Figure 3.4 Rumen pH of growing wether lambs offered diets *ad libitum*

3.3.5 Rumen Degradability And Synchrony Index

The hourly intake pattern of each animal was run through the SIRE programme in order to predict the amount of OM, carbohydrate and N degraded in each diet and to examine the synchrony index according to the VFI selected by the animals. These results are presented in Table 3.11. The amount of OM degraded was significantly lower ($p<0.01$) for the grass silage diets (G and GS). However, the addition of the synchronous supplement significantly increased ($p<0.001$) the degradation of the OM in the rumen.

The degradation of carbohydrate in the rumen was significantly greater ($p<0.001$) when animals were fed the grass silage diets than when fed diet A and there was a large increase in carbohydrate degradability when the asynchronous diets were supplemented.

The amount of N degraded in the rumen was similar for diets A and AS and these values were significantly greater ($p<0.001$) than predicted quantity of N released when animals were fed the grass silage diets. The difference between the two asynchronous diets (G and A) was greater than between the more synchronous diets.

When the synchrony index of OM degradability was examined according to the intake pattern selected by the animals, a significant difference ($p<0.001$) was seen between the grass silage diets (G and GS) and diets A and AS. When the actual synchrony indices were compared to those predicted, it can be seen that the animals were able to select a more synchronous diet when offered the grass silage diet. However, the synchrony of nutrient release in Diets GS, A and AS was lowered by the pattern of intake selected, especially that of diet A.

Table 3.11 Actual amounts of OM, CHO and N degraded (g/day) and synchrony index of diets (determined by recording *ad libitum* food intake patterns and translating through SIRE)

	Treatment Means					Significance of main effects		
	G	GS	A	AS	s.e.d.	S	C	I
OM deg'd	626	956	819	998	43.8	**	***	*
CHO deg'd	279.6	576.1	168.1	518.9	22.67	***	***	NS
N deg'd	25.26	32.70	39.42	37.08	1.716	***	(NS)	***
Synchrony Index (OM)	0.3525	0.7225	0.2350	0.6438	0.01924	***	***	NS
Predicted	0.30	0.85	0.51	0.88				

3.4 DISCUSSION

3.4.1 Daily Intake And Whole Tract Digestibility

The total daily DMI of the four feeds resulted in the grass silage diet having a much lower intake than that of the supplemented silage. Both grass silage diets were consumed in significantly lower ($p<0.001$) amounts than either diets A or AS, which had similar DM intakes. The results suggest that the presence of fermentation products present in the grass silage appeared to have a major effect on DMI, as the greatest daily intake is observed in those diets which include no fermentation products. However, it should also be noted that the physical form differed between the diets based on grass silage, where a chopped forage was fed, compared with those based on diet A, where a ground forage was offered. The effect of these fermentation products to decrease intake in comparison to the fresh product, due to factors such as VFAs, amines and ammonia, aswell as a low pH and DM is well documented (Clancy *et al*, 1977). Physical limitation is also known to have an effect (Farhan and Thomas, 1978) and may have contributed to the reduction in intake, due to rumen fill and

increased rumination time. These factors appeared to have an effect on the intake of the animals in this study.

Improving synchrony, in terms of hourly nutrient supply to the rumen, had a significant effect ($p<0.01$) on daily intake, although it was only the intake of the grass silage (G) that was increased when the supplement was added. This may have been due to the diet being easier to consume, or less fermentation products being present, rather than the synchrony directly, as diet GS contained only half the amount of grass silage as diet G (in terms of DM) and there was no increase in intake when diet A was supplemented.

Values for DM digestibility showed an inverse trend to those for DMI. This may have been a result of rumen outflow rates, with the grass silage diets, which contained long fibre, being expected to be retained in the rumen longer (Dulphy and Demarquilly, 1973; Murdoch, 1965; Wilkinson *et al*, 1978). This would have resulted in more rumination and therefore more degradation than diet A, in which the ingredients were ground, resulting in increased digestibility. Forbes (1995) found that grinding feed resulted in an increase in the rate of passage and that this decreased time spent in the digestive tract, resulting in an increased VFI, but there was a decrease in digestibility.

El-Shazly *et al* (1961) supplemented roughages with readily-fermentable energy sources and found a decreased extent and rate of crude fibre digestion, a result in accordance with the effects of supplementing either diet G or A with a rapidly fermented carbohydrate source in the current work. When Morgan *et al* (1979) supplemented grass silage with a barley supplement, they found a lower DMI of the silage and the digestibility of the cell wall

constituents was decreased compared to the silage fed alone. This effect was attributed to the effect of starch yielding acidic conditions in the rumen which are unsuitable for fibre digestion.

NDF digestibility was found to be significantly greater ($p<0.001$) when animals were fed the silage diets, (G and GS) which is likely to be due to rumen retention time. However, improving the hourly synchrony of the release of nutrients in the rumen for either diet G or A resulted in the digestibility of NDF decreasing.

The digestibility of OM in animals fed the grass silage diets was also greater than that of diets A and AS. When the grass silage was supplemented to improve synchrony, the digestibility of the OM was significantly reduced ($p<0.05$). However, when diet A was supplemented, OM digestibility improved, suggesting that sychronising the release of nutrients may be of benefit to this parameter for this diet. This current finding is in contrast to Morgan *et al* (1979) who found that the digestibility of OM increased when a barley (and soyabean) supplement was fed with the grass silage.

When animals were offered the grass silage diets (G and GS), the N digestibility was significantly higher ($p<0.001$) than when the diets A and AS were offered. N digestibility of the synchronous diets was significantly lower ($p<0.01$) than in the asynchronous diets. Work by Raymond *et al* (1986) showed that the addition of a starchy supplement reduces the digestibility of a diet, due to a lowering of rumen pH which reduces microbial efficiency.

The higher digestibility of DM, OM and NDF of the grass silage could be linked to rumen

pH, which was found to remain higher when this diet was offered, allowing rumen microbes to be more efficient. Previous work by Carro *et al* (1994) has shown that microbes are most effective at a pH greater than 6.5, and rumen pH was found not to fall below this level when animals were fed the grass silage in this study.

3.4.2 Intake Pattern

The differences in food intake between diets was found shortly after feeding, when the main meal of the day was consumed. Table 3.6 showed that, although the animals were fed *ad libitum*, the greatest food intake was observed immediately after fresh food was offered.

Although the rate of eating was not measured, it can be related to the amount eaten over a defined length of time. Forbes *et al* (1972) found that sheep ate significantly faster during the first 30 minutes after being offered fresh food, and this effect was more apparent when animals were fed on ground food rather than on long hay. The rate of eating of unground diets ie. long food, is negatively related to its retention time and positively related to total VFI. In the study of Forbes *et al* (1972), there was no significant difference in rate of eating in the second and third 30 minute block.

The more synchronous diets continued to be consumed into the second hour after feeding, with significantly larger ($p<0.001$) intakes than the asynchronous diets being observed. This follows the suggestion that synchronous diets could be taken in one large meal, whereas asynchronous diets must be taken in smaller, more discrete meals to take account of the differences in the rate of release of nutrients.

Studies by Gill *et al* (1988) and Thiago *et al* (1992) showed that the intake pattern of cattle fed silage is characterised by a relatively short main meal and a high number of short meals through the day. Dulphy (1985) proposed that sheep compensate for a lower intake during the main meal of the day by taking more meals subsequently during the day or by increasing their size, whilst Dulphy and Van Os (1996) reported that, with silage, the shorter duration of the main meal and subsequent small meals are partly a consequence of a lower palatability.

Thiago *et al* (1992) also reported that the intensity of rumen contractions in animals offered grass silage was minimal for the first 9 hours after feeding, but increased thereafter. There was higher activity in the second half of the 24 hour feeding cycle, perhaps due to a greater disappearance of digesta from the rumen. The peak outflow of indigestible OM and indigestible NDF occurred 16-20 hours after feeding, which corresponded with the peak contractile activity (Thiago *et al*, 1992). Similarly, both Harris and Phillipson (1962) and Phillips and Dyck (1964) found an increased flow during the second half of the feeding cycle.

Gill and Romney (1994) discounted the hypothesis that factors limiting the size of the first meal dominate the control of daily intake, based on eating patterns for forages. Instead, they proposed that intake 'drive' is related to energy/oxygen metabolism of the animal, and meal size and pattern may be controlled to minimise production of waste products.

The similar patterns of intake can be observed in Table 3.6. Hourly DMI decreased until hour 6, when there was an increase in feeding activity which continued in all treatments for the following seven hours before decreasing, although the silage diets showed less variation between hours towards the end of this period. This was illustrated by a significant difference

($p<0.05$) between diets G and A at hour 11. DMI was relatively low through the night, with little feeding activity until hour 23 when the intake of diet AS significantly increased ($p<0.05$) when animals may have anticipated the arrival of fresh food. Johnson and Combs (1992) observed that changes in feeding behaviour or rumen activity have enabled cows to accommodate extra fill without decreasing intake.

When the hourly DMI was examined as a proportion of total daily DMI, the first hour of the the day was found to account for the greatest proportion of the daily intake being consumed in all treatments. However, during hour 2, the proportions of the more synchronous diets consumed were significantly greater ($p<0.01$) than those of the asynchronous diets due to continued eating. Although Table 3.7 shows no significant differences ($p\geq0.05$) through the day, due to similar patterns of intake proportions, Figure 3.1 illustrates that the more synchronous diets tended to have a more variable intake than the asynchronous diets when compared by silage type. Diet G was consumed in meals of size that change gradually compared to diet GS, which had a variable intake, especially between hours 7 and 8. However, this pattern was more noticeable when diets A and AS were compared, particularly between hours 6 and 11. The difference in proportion size between the highest and lowest hourly proportions of the two diets is greatest for diet A.

Dulphy (1985), Chiofalo *et al* (1992) and Thiago *et al* (1992) found that animals consume one short principal meal after the feed is offered, then a relatively high number of discrete meals throughout the day. Van Os *et al* (1995) found that with a silage diet, the principal meal accounts for 22% of daily DMI, whilst Dulphy (1985) reported that intake-regulating mechanisms have less effect over a daily period than over the short period of the principal

meal. Chiofalo *et al* (1992) found that there was an increase in rumen load during the day, therefore termination of the principal meal was not due to a limitation of rumen capacity.

The comparison of accumulative hourly intake is distorted by the significant differences ($p<0.01$) in total daily DMI to some extent, as the main effects become more significant as the hour post-feeding increases. Diets G and GS had lower hourly intakes than diets A and AS throughout the day. The grass silage had a steadier rate of intake than other diets. Figure 3.1 also illustrates that the asynchronous diets seemed to be consumed in a more steady pattern than the more synchronous diets. This is clearer when diets A and AS are compared. Although the intake in hour 1 and the total daily DMI were very similar for both diets, diet AS was eaten faster in the earlier part of the day, whereas diet A was eaten in a steadier, more regular pattern of intake.

When the hourly DMI is blocked into sections through the day, the results show that improving the synchrony of diets G and A significantly increased ($p<0.05$) intake in the first block of all aggregated intakes, although this difference between treatments decreased as the number of hours in the block increased. The intake of grass silage diets was generally lower than that of diets A and AS when compared in this blocked form, although this relates to total daily DMI.

3.4.3 Blood Metabolites

Blood plasma β-hydroxybutyrate (BHB) concentration indicates the level of butyrate absorbed from the rumen, and to some extent indicates the amount of energy available for body metabolism (McDonald *et al*, 1988). When animals were offered the grass silage diets,

the level of BHB was greater than diets A and AS until hour 15, after fresh feed was offered, although the difference was only significant ($p<0.05$) when animals were sampled three hours after feeding.

In samples taken from fifteen hours post-feeding onwards, the concentration of BHB was higher for the animals fed the diets A and AS. The main effect on plasma BHB concentration was observed by supplementing either diet G or A with the concentrate. From hour 3 onwards, the concentration of plasma BHB was elevated by feeding a more synchronous diet (GS and AS). This suggests that there is an improvement in absorbtion of butyrate from the rumen when nitrogen and energy are available simultaneously and would have economic implications for growing animals.

The plasma urea pool is a 'reserve N pool', and when N is in excess in the rumen, ammonia N is absorbed and appears as urea in the plasma urea pool. This N may be recycled back to the rumen during subsequent periods of N shortage (McDonald *et al*, 1988). N recycling may explain the lack of response to better synchronisation of energy and N supply to the rumen (Henning *et al*, 1993). However, this may not hold at higher levels of energy and N input - the excess absorbed N may be excreted in urine and recirculation of N to the rumen at a later stage may be insufficient to meet the needs required by the higher energy supply.

Houpt (1970) and Kennedy and Milligan (1980) found that the recycling of urea to the rumen is positively related to the amount of soluble carbohydrate entering the rumen or to the rate of OM fermentation in the rumen. In agreement, Cocimano and Leng (1967) reported that recycling of urea to the rumen was positively related to the amount of soluble carbohydrate

entering the rumen or to the rate of OM fermentation in the rumen. In contrast, Henning *et al* (1993) reported that if the overall balance between ruminally available N and ruminally fermentable OM in daily intake is sufficient, there is no further advantage in synchronising release of energy and N in the rumen over the shorter term.

3.4.4 Rumen pH

Mould and Orskov (1984) found that by decreasing rumen pH to 6.0 - 6.1 in sheep given roughage that cellulolysis was inhibited due to partial destruction of the rumen microflora. This resulted in decreased DM degradation, resulting in lower DMI. Decreased cellulolysis, caused by decreased pH, due to less rumination, resulted in decreased buffering capacity and rapid microbial fermentation.

Maintaining a constant intake gives a flow of saliva to the rumen, resulting in buffering of pH levels (Webster, 1987), suggesting that the pattern of intake of the grass silage and the higher values of pH seen in diet G (Figure 3.4) may be a consequence of each other.

Decreased cellulolytic activity is seen at pH 6.2 - 6.3, and the degradation of hay was depressed from 30% to 9% when rumen pH was lowered from pH 6.6 to 6.0 (Carro *et al*, 1994). Cellulolysis is partially inhibited if pH is taken from 6.6 to 6.2, and totally inhibited at less than pH 6.0. This inhibition results in a decreased intake. In contrast, supplementation significantly reduced ($p<0.05$) rumen pH, an effect associated with a reduction in fibre digestion.

Rumen pH was significantly higher ($p<0.05$) in the fresh silage diets, which is likely to be due to the increased amount of rumination in comparison to the synthetic diets, as the fibre is

longer. The presence of the starchy concentrate tended to lower pH, with an interaction being present due to a greater effect in the fresh silage diet. However, this may be related to the amount of silage and long fibre in the diet.

Van Os *et al* (1995) found that, generally, rumen pH decreased by 0.3 pH units during the principal meal and remained relatively constant at 6.6 during the day. In the current work, rumen pH was similar throughout the day in lambs fed diet G or A.

3.4.5 Rumen Degradability And Synchrony Index

When animals were offered the diets *ad libitum*, the synchrony index decreased for all treatments except the grass silage diet. It appears, therefore, that the animals were not capable of selecting a pattern of intake which resulted in a more synchronous release of nutrients in the rumen than the predicted value.

3.5 CONCLUSIONS

VFI is controlled by a combination and additive effect of physical and chemical signals which give a total signal to the central nervous system (CNS) resulting in satiation (Forbes, 1980). In the case of forage foods, the physical signals are stronger than the chemical signals, so that the chemical signal to induce satiation is relatively weak (Forbes, 1995). This would prioritise physical fill and the effect of rumen distension over the effect of fermentation products. Although both factors have an important role in the control of VFI of grass silage, in this study the physical factor of bulk may be the main controlling factor, but is triggered by the negative effect of the presence of silage fermentation products, which provided a weaker signal.

CHAPTER 4

4 INFLUENCE OF ALTERING THE PATTERN OF NUTRIENT RELEASE FROM GRASS SILAGE BY THE ADDITION OF SUPPLEMENTS VARYING IN SYNCHRONY INDEX

4.1 INTRODUCTION

Results from the previous study indicated that the physical presence of grass silage in a diet was the major influence in altering the VFI of growing wether lambs, which tended to overshadow the influence of altering the synchrony of hourly release of nutrients in the rumen, although VFI pattern was altered to some extent. The aim of the present study was to base all diets on the same grass silage, but to add a supplement which would alter the synchrony index of the complete diet, so that this factor of VFI could be more closely examined. This would result in four diets which were similar in DM content, ME and CP, but varied in their hourly rate of release of nutrients in the rumen.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Four Suffolk wether sheep, aged *c.*12 months, weighing *c.*60kg and fitted with permanent rumen cannulae were housed in individual sawdust-bedded pens. Housing was continuously lit. Animals were weighed on days 1, 8 and 24 at 1400h.

4.2.2 Diets

All animals were fed a complete diet consisting of the grass silage, described in section 2.1.3, plus a supplement, at a rate of 50:50 in terms of DM. The supplements were formulated to differ in the hourly rate of release of nutrients and, therefore, the degree of synchrony of

these nutrients. Diets were formulated using the SIRE diet formulation computer package (based on known degradability coefficients, assuming a rumen outflow rate of 0.05 and that the 1kg of feed offered per day was totally consumed in the first hour after feeding), to be similar in terms of ME (MJ/kg DM) and CP (g/kg DM). Table 4.1 presents the formulations of each supplement, and Table 4.2 their predicted chemical composition.

Table 4.1 Dietary composition (g/kg DM) for four supplements, differing in synchrony of hourly nutrient supply to the rumen, to be fed with grass silage (50:50 on a DM basis) as a complete diet to wether sheep

Ingredient	Supplement			
	Slow Energy, Fast N	Fast Energy, Fast N	Slow Energy, Slow N	Slow Energy, Fast N
Wheat straw	250	240	260	220
Barley	---	556	---	590
Malt Distiller's Dark Grains	160	160	---	20
Rapeseed Meal	---	---	170	170
Fishmeal	---	30	---	---
Sugar beet pulp (unmolassed)	540	---	538	---
Urea	12	3	6	---
Fat	38	11	26	---

Table 4.2 Predicted chemical composition (g/kg DM) for four supplements, differing in synchrony of hourly nutrient supply to the rumen

Ingredient	Supplement			
	Slow Energy, Fast N	Fast Energy, Fast N	Slow Energy, Slow N	Slow Energy, Fast N
CP (g/kg DM)	143	146	147	146
FME (MJ/kg DM)	9.48	10.28	10.05	10.99
MP (g/kg DM)	87.3	89.4	95.2	90.9
ERDP/FME	8.4	9.1	7.1	8.2
Daily ratio of N:OM	35	30	30	27
Synchrony index	0.53	0.86	0.90	0.34

The complete diets were mixed immediately prior to feeding and animals were offered their allocated diet *ad libitum* at a rate of 115% of appetite. Refusals were collected daily and intake requirements calculated for the following day. Animals were fed once daily at 9.30 am.

Table 4.3 presents the basic formulation of the complete diets and the names used for these diets during the study.

Table 4.3 Basic formulation, names and abbreviations used for complete diets

	Synchrony of complete diet	Name of complete diet
Grass silage + Supplement Slow Energy, Fast N	Asynchronous	ASYN
Grass silage + Supplement Fast Energy, Fast N	Intermediate	INT(FE)
Grass silage + Supplement Slow Energy, Slow N	Intermediate	INT(SE)
Grass silage + Supplement Fast Energy, Slow N	Synchronous	SYN

The predicted chemical composition, degradability and synchrony indices of the four complete diets are presented in Table 4.4.

Table 4.4 Predicted chemical composition (g/kg DM), degradability and synchrony index of four complete diets (fed silage:supplement at 50:50 on a DM basis)

	ASYN	INT(FE)	INT(SE)	SYN
ME (MJ/kg DM)	11.46	11.52	11.45	11.55
CP (g/kg DM)	155.55	157.0	158.0	157.0
FME (MJ/kg DM)	8.79	9.19	9.08	9.55
MP (g/kg DM)	82.95	82.10	91.15	86.00
ERDP/FME	11.5	11.7	10.7	11.1
Daily ratio of N:OM (gN/kgOM)	38	35	36	33
Synchrony index	0.45	0.64	0.64	0.88
OM(g/kg DM)	893.82	908.51	898.18	921.89
Total CHO	526.03	575.41	534.74	606.43
Starch	1.42	74.11	1.59	136.42
W-S CHO	32.46	34.97	40.48	33.01
NDF	510.48	475.50	509.04	437.01
ADF	287.35	265.74	287.80	236.35
Hemicellulose	223.11	209.72	221.21	200.61
Cellulose	248.89	224.19	243.85	198.25
Ether extract	47.99	37.64	38.30	29.32
ERDP	115.28	112.89	98.05	109.20
DUP	26.21	27.26	44.02	35.92

Table 4.5 Predicted degradability coefficients and synchrony index of four complete diets (fed silage:supplement at 50:50 on a DM basis)

	ASYN	INT(FE)	INT(SE)	SYN
OM	0.505	0.544	0.495	0.575
CHO	0.174	0.261	0.176	0.331
N	0.022	0.021	0.018	0.020
Ratio total gN/kgOM/d	43.64	38.87	37.06	35.51
Ratio total gN/kgCHO/d	126.59	80.90	104.19	61.66
N degradability	0.801	0.785	0.676	0.743

Water and mineral blocks were available at all times. Each 25kg batch of supplement was sampled prior to analysis. The grass silage was sampled weekly. All samples were frozen until analysis.

4.2.3 Experimental Procedure

A 4 x 4 Latin square design was used, with each animal spending one period on each diet. Each period lasted for 32 days - 14 days adaptation, including 4 days changeover, followed by an 18-day sampling period. A 6-day faecal collection was carried out on days 15-21, following the method described in section 3.2.3.

On days 21-24 polysynthetic fibre bags were used to examine the rumen degradability of each diet. Approximately 5g DM of each diet was weighed into each of six bags. The grass silage was roughly chopped to 1cm with a food processor and the concentrate ground through a 2mm screen then sieved through a 42μm mesh. Silage and concentrate were weighed into the dacron bags separately and then thoroughly mixed. These were suspended in the rumen by

insertion through the rumen cannulae 30 minutes after the feed was offered. The bags containing feed samples were removed at 2, 4, 8, 12, 24 and 48 hours after insertion. After removal, the bags were washed in the cold water cycle of a washing machine. To estimate the immediately soluble fraction of each diet, bags containing a sample of each diet were put through the cold water rinse. The residue from all samples was oven-dried for 48 hours at 70°C until constant weight. The feed residues were analysed for N and OM and the cellulose residues weighed, as described in section 2.1.2.4.

On days 25-28, voluntary food intake was recorded as described in section 3.2.3 with the data logger set to record the voltage from each balance at 1 minute intervals. On collecting the data from the logger and video, the hourly intake of each animal was calculated by the addition of sixty 1-minute readings starting at the time of feeding. The number of meals over the 24-hour period was calculated from the video. A meal was defined as an animal eating for at least 2 minutes and a break of more than 4 minutes was considered to be an inter-meal period. Therefore, if the animal stopped eating for less than 4 minutes during its meal this was considered to be a break within the meal (Forbes, 1995). From these results, the total time spent eating during the 24-hour period, the average meal size and average rate of eating were calculated.

Blood sampling was carried out at 7 time-points on days 28 (1330h and 1930h), 29 (1030h and 1630h) and 30 (0830h, 1130h and 2230h) of each period, following the method described in section 3.2.3.

Rumen sampling was carried out at 7 time-points on days 31 (1030h), 32 (0830h, 1130h,

1430h, 1730h and 2030h) and 33 (0430h) via the rumen cannulae using a hand-operated vacuum pump. On extraction, the fresh rumen fluid was treated as described in section 3.2.3. The pH was taken and a subsample (10ml) from these time-points was frozen immediately, without being acidified, for subsequent measurement of osmolality (section 4.2.4.2).

Table 4.6 Experimental procedure for wether sheep fed diets based on grass silage with four supplements differing in their pattern of nutrient release in the rumen

Day	Procedure
1	Animals weighed
1 - 4	Diet changeover period
1 - 14	Adaptation period
8	Animals weighed
14	Attach faecal harnesses
15 - 21	Faecal collection
21 - 24	Rumen degradability work
24	Set up data logger and video
	Animals weighed
25 - 28	Record voluntary food intake pattern
28 - 30	Blood sampling
31 - 32	Rumen sampling

4.2.4 Chemical Analysis

4.2.4.1 Analysis of Feed, Faecal and Blood Samples

All feed and faecal samples were oven dried and ground through a 1mm screen (Retsch ZM 1000) prior to proximate analysis. Each sample was analysed in duplicate for dry matter, organic matter, nitrogen and NDF as described previously. Additionally, feed samples were

analysed for ADF and ether extract. Blood plasma samples were analysed for urea and BHB.

4.2.4.2 Osmotic Pressure

The rumen fluid samples were stored frozen until required, then thawed at room temperature. To remove the solid phase from the rumen fluid, a Sorville RC-5B refrigerated centrifuge was used to spin the samples at 5000rpm for 15 minutes. The supernatant was measured in duplicate for osmotic pressure on an Osmette Model 2007 (Precision Instruments).

4.2.5 Statistical Analysis

Results were evaluated by analysis of variance (ANOVA) using the statistical package Genstat 5 (Lawes Agricultural Trust, 1987). Results were analysed to identify any effects of the degree of synchrony of nutrient release in the rumen and sub-divided into supplement type:

	E	-	fast energy v slow energy
	N	-	fast nitrogen v slow nitrogen
	Int	-	interaction between supplements (rate of energy x N)

4.3 RESULTS

4.3.1 Daily Intake And Whole Tract Digestibility

Results for the mean values of DMI, NDF, OM and N digestibility are presented in Table 4.7. There were no significant differences ($p \geq 0.05$) observed in the total daily DMI of the four diets. Diet SYN had the lowest daily DMI and diet INT(FE) had the highest DMI, but the range in intake values was limited.

Table 4.7 Daily DMI (kg/day) and whole tract digestibility coefficients of DM, NDF, OM and N.

	Treatment Means					Significance of main effects		
	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
DMI	3.167	3.294	3.186	3.138	0.142	NS	NS	NS
DM dig	0.8295	0.8362	0.8210	0.8393	0.01260	NS	NS	NS
NDF dig	0.8175	0.7800	0.8260	0.7698	0.01604	**	NS	NS
OM dig	0.8372	0.8430	0.8400	0.8458	0.01226	NS	NS	NS
N dig	0.8458	0.8648	0.8393	0.8580	0.01118	NS	NS	NS

GS = grass silage

The DM digestibility of all four diets was very similar, with no significant differences ($p \geq 0.05$) observed.

NDF digestibility was significantly higher ($p<0.01$) in grass silage diets supplemented with concentrate where the energy source was slowly degradable in the rumen. The rate of release of N supplied by the concentrate supplement had no significant effect ($p \geq 0.05$) on N digestibility.

There were no significant differences ($p \geq 0.05$) in the OM digestibility of the four diets, with the observed values being very similar.

The digestibility of N was found to be slightly higher in grass silage diets supplemented with a concentrate that supplied a rapidly degradable energy source, although the differences between diets were not significant ($p \geq 0.05$).

4.3.2 Degradability Characteristics

The degradability coefficients of the four diets are presented in Table 4.8.

Table 4.8 Degradability coefficients of complete diets offered to wether sheep

Component	'a'	'b'	'c'	'a' + 'b'	lag	r^2
DM:						
ASYN	0.536	0.312	0.1127	0.848	1.99	98.2
INT(FE)	0.566	0.286	0.0844	0.852	0.00	94.8
INT(SE)	0.506	0.339	0.1130	0.845	2.30	96.6
SYN	0.549	0.290	0.1030	0.839	0.00	97.3
s.e.d.	0.0039	0.0124	0.01396	0.0108	0.297	
N:						
ASYN	0.731	0.195	0.1257	0.926	---	92.9
INT(FE)	0.748	0.176	0.1747	0.924	---	86.5
INT(SE)	0.660	0.266	0.0978	0.926	---	91.8
SYN	0.686	0.236	0.2187	0.922	---	95.6
s.e.d.	0.0047	0.0110	0.05690	0.0086	---	
OM:						
ASYN	0.514	0.332	0.1103	0.846	1.99	98.2
INT(FE)	0.544	0.308	0.0793	0.852	0.00	95.7
INT(SE)	0.480	0.361	0.1115	0.841	2.29	96.6
SYN	0.526	0.308	0.1040	0.834	0.00	97.2
s.e.d.	0.0115	0.0185	0.01300	0.0108	0.742	

The results showed that diets were similar in their 'a' + 'b' values (the total of the soluble fraction + the potentially degradable fraction) within each component characterised. However, the diets varied in their individual 'a' and 'b' values within the components, with regards to the proportions of these coefficients.

The values for the 'a' fraction of the DM of the four diets were greater when the energy source supplied by the concentrate was rapidly-degradable in the rumen, but these diets had lower values for the potentially degradable fraction ('b') of DM. The rates at which the potentially degradable fraction of the DM was degraded ('c') were greater when the energy source supplied by the concentrate was slowly degradable. A lag was present for the degradation of DM only when the concentrate supplied a slowly-degradable energy source.

The degradability of N in the diets appeared to be dependent on its predicted rate of release in the rumen in terms of its 'a' and 'b' values. The 'a' + 'b' coefficients were similar for the four diets, but the proportion of the soluble fraction ('a') was greater for diets where the N in the supplement was predicted to be rapidly degraded. When the N in the supplement was slowly degraded in the rumen, the potentially degradable fraction ('b') of N was greater. The rate of degradation of the potentially degradable N ('c') was considerably greater for diet SYN in comparison to other diets, with diet INT(SE) having a slower rate of N degradability in the rumen.

The values for the degradability coefficients of OM were similar for all four diets in terms of their soluble and potentially degradable fractions ('a', 'b' and 'a' + 'b'). Diet INT(FE) had a considerably slower rate of degradability of the 'b' fraction compared to other diets, with diets where the concentrate supplied a slowly-degradable source of energy having a higher 'c' value. There was no lag present for OM degradation in diets where the concentrate supplied a rapidly-degradable energy source. However, the lag values for diets ASYN and INT(SE) had lags present which were similar to those present for DM.

The degradability coefficients presented for the characterisation of DM and OM showed similar trends across their values for all diets.

4.3.3 Intake Pattern

Table 4.9 presents the hourly DMI of the four diets. Results show that DMI was greatest during the first hour after fresh feed was offered. Most feeding occurred during the first 12 hours after feeding, with intakes being fairly constant, although increased feeding activity was observed during hours 5 and 6. There were no significant differences ($p \geq 0.05$) observed between the diets, except at hour 16, when grass silage supplemented with a concentrate that supplied a rapidly-degradable N source had significantly higher ($p<0.05$) intakes. The similar patterns of feed intake are illustrated in Figure 4.1. There was little feeding activity observed after hour 14, until fresh feed was offered.

Table 4.9 Hourly food intake pattern (kg DM)

	Treatment Means					Significance of main effects		
Hour	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
1	0.695	0.679	0.670	0.600	0.0732	NS	NS	NS
2	0.165	0.188	0.209	0.211	0.0656	NS	NS	NS
3	0.158	0.103	0.119	0.216	0.0741	NS	NS	NS
4	0.165	0.150	0.194	0.180	0.0785	NS	NS	NS
5	0.214	0.262	0.128	0.200	0.0875	NS	NS	NS
6	0.268	0.278	0.250	0.284	0.0785	NS	NS	NS
7	0.054	0.120	0.133	0.152	0.0617	NS	NS	NS
8	0.187	0.272	0.185	0.173	0.0987	NS	NS	NS
9	0.174	0.120	0.139	0.157	0.1117	NS	NS	NS
10	0.050	0.160	0.117	0.072	0.0935	NS	NS	NS
11	0.220	0.121	0.113	0.166	0.0888	NS	NS	NS
12	0.188	0.210	0.116	0.198	0.0547	NS	NS	NS
13	0.184	0.186	0.231	0.106	0.0450	NS	NS	NS
14	0.044	0.027	0.039	0.003	0.0284	NS	NS	NS
15	0.142	0.142	0.098	0.115	0.0432	NS	NS	NS
16	0.129	0.148	0.060	0.047	0.0456	NS	*	NS
17	0.087	0.073	0.054	0.155	0.0574	NS	NS	NS
18	0.100	0.017	0.044	0.035	0.0531	NS	NS	NS
19	0.041	0.099	0.074	0.046	0.0436	NS	NS	NS
20	0.021	0.006	0.036	0.005	0.0154	NS	NS	NS
21	0.018	0.068	0.036	0.087	0.0390	NS	NS	NS
22	0.037	0.011	0.014	0.061	0.0424	NS	NS	NS
23	0.000	0.003	0.000	0.003	0.0031	NS	NS	NS
24	0.014	0.027	0.046	0.046	0.0323	NS	NS	NS

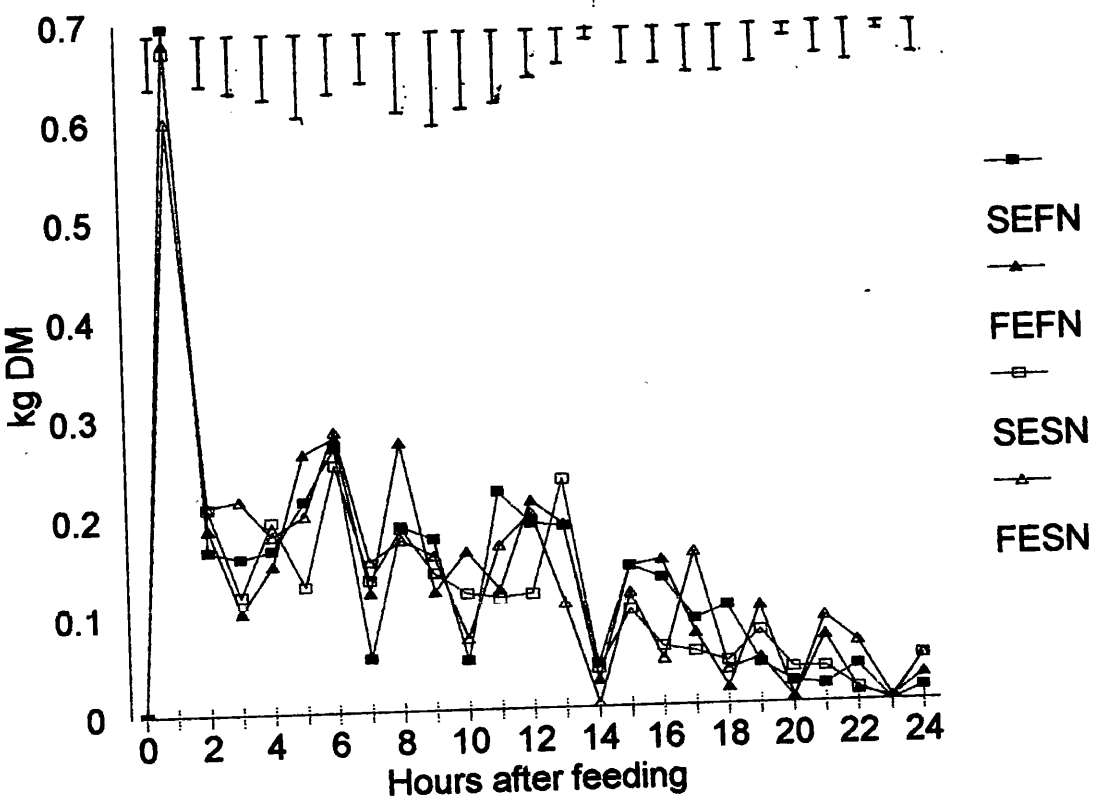

Figure 4.1 Hourly food intake pattern (kg DM) of wether sheep when offered four complete diets differing in their pattern of nutrient release in the rumen

When hourly DMI is presented as a proportion of total daily DMI, the pattern of intake appears similar throughout the day, with few significant differences observed in Table 4.10. The large proportion of the daily DMI consumed during hour 1 after feeding is illustrated, accounting for 18.20 - 20.98% of the total daily intake in all four diets.

During hour 16, there was a significantly greater ($p<0.05$) intake in terms of proportion of total daily DMI for grass silage supplemented with concentrate supplying a rapidly-degradable source of N.

Table 4.10 Hourly food intake (g DM as % of total daily DMI)

	Treatment Means					Significance of main effects		
Hour	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
1	20.14	19.37	20.98	18.20	1.688	NS	NS	NS
2	4.73	5.13	7.08	6.46	1.935	NS	NS	NS
3	4.52	3.36	3.39	6.43	2.386	NS	NS	NS
4	5.29	4.75	6.53	5.42	2.352	NS	NS	NS
5	5.67	7.39	4.25	5.75	2.265	NS	NS	NS
6	8.04	8.27	8.26	8.75	1.950	NS	NS	NS
7	1.85	3.37	3.40	4.57	1.398	NS	NS	NS
8	5.91	7.99	6.84	5.48	2.792	NS	NS	NS
9	4.60	3.80	4.00	5.00	2.860	NS	NS	NS
10	1.49	4.60	3.96	2.05	2.661	NS	NS	NS
11	7.03	2.74	3.62	5.26	2.261	NS	NS	NS
12	6.15	5.71	3.78	5.80	1.645	NS	NS	NS
13	5.02	5.69	7.00	3.04	1.534	NS	NS	NS
14	1.62	0.65	1.42	0.09	1.098	NS	NS	NS
15	4.58	4.24	3.14	3.46	1.326	NS	NS	NS
16	3.72	4.13	1.94	1.35	1.114	NS	*	NS
17	2.51	2.30	2.07	4.44	1.424	NS	NS	NS
18	2.91	0.56	1.54	1.12	1.503	NS	NS	NS
19	1.38	2.96	2.12	1.59	1.368	NS	NS	NS
20	0.60	0.19	1.18	0.17	0.467	NS	NS	NS
21	0.63	1.60	1.21	2.59	0.856	NS	NS	NS
22	1.13	0.24	0.50	1.67	1.206	NS	NS	NS
23	0.00	0.10	0.00	0.08	0.100	NS	NS	NS
24	0.47	0.88	1.75	1.25	1.121	NS	NS	NS

The results of hourly DMI on an accumulative basis are presented in Table 4.11. There were no significant differences ($p \geq 0.05$) observed between diets and no differences in intake

pattern can be observed from the results.

Table 4.11 Hourly accumulative food intake pattern (kg DM)

	Treatment Means					Significance of main effects		
Hour	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
1	0.695	0.679	0.670	0.600	0.0732	NS	NS	NS
2	0.859	0.866	0.879	0.811	0.1064	NS	NS	NS
3	1.017	0.968	0.999	1.027	0.1219	NS	NS	NS
4	1.182	1.119	1.192	1.207	0.1167	NS	NS	NS
5	1.396	1.380	1.320	1.407	0.1348	NS	NS	NS
6	1.664	1.658	1.570	1.690	0.1885	NS	NS	NS
7	1.718	1.778	1.703	1.843	0.1728	NS	NS	NS
8	1.905	2.050	1.888	2.017	0.1496	NS	NS	NS
9	2.080	2.171	2.027	2.174	0.2411	NS	NS	NS
10	2.130	2.330	2.144	2.245	0.2055	NS	NS	NS
11	2.350	2.451	2.257	2.411	0.2320	NS	NS	NS
12	2.537	2.662	2.374	2.609	0.1984	NS	NS	NS
13	2.721	2.847	2.605	2.715	0.1912	NS	NS	NS
14	2.765	2.874	2.643	2.718	0.1825	NS	NS	NS
15	2.907	3.016	2.741	2.833	0.2109	NS	NS	NS
16	3.036	3.164	2.801	2.880	0.2188	NS	NS	NS
17	3.123	3.237	2.855	3.035	0.1713	NS	NS	NS
18	3.223	3.254	2.899	3.070	0.2022	NS	NS	NS
19	3.264	3.352	2.974	3.116	0.1727	NS	NS	NS
20	3.285	3.358	3.010	3.121	0.1757	NS	NS	NS
21	3.303	3.425	3.045	3.208	0.1888	NS	NS	NS
22	3.340	3.436	3.059	3.269	0.1629	NS	NS	NS
23	3.340	3.439	3.059	3.272	0.1622	NS	NS	NS
24	3.354	3.466	3.105	3.318	0.1414	NS	NS	NS

Blocking DMI into periods of greater than 1 hour gave slightly different results to examining DMI on an hourly basis. Table 4.12 shows the results of the hourly DMI being blocked into 4, 6, 8 and 12 hour sections. Animals offered grass silage supplemented with concentrate supplying a rapidly-degradable source of energy had significantly greater ($p<0.05$) intakes during the second 4-hourly block. There was also a significant increase ($p<0.05$) in intake in the diets supplemented with concentrate supplying a rapidly-degradable source of N during the fourth 4-hourly block, especially with supplement SYN.

Blocking hourly DMI into 6-hourly sections showed no significant differences ($p\geq0.05$) between diets.

When DMI was blocked into 8-hourly sections, a significant difference ($p<0.01$) was observed during the second 8-hourly block. Diets supplemented with concentrate supplying a rapidly-degradable source of N had significantly higher intakes than those with a slowly-degradable N source.

No significant differences ($p\geq0.05$) were observed between intakes of diets when DMI was blocked into 12-hourly sections.

Table 4.12 Blocked food intake pattern (kg DM)

	Treatment Means					Significance of main effects		
Hours	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
1 - 4	1.182	1.120	1.195	1.205	0.1173	NS	NS	NS
5 - 8	0.722	0.932	0.698	0.807	0.0770	*	NS	NS
9 - 12	0.633	0.610	0.485	0.595	0.0766	NS	NS	NS
13 - 16	0.498	0.503	0.428	0.270	0.0632	NS	*	NS
17 - 20	0.248	0.193	0.208	0.243	0.0901	NS	NS	NS
21 - 24	0.068	0.108	0.098	0.198	0.0745	NS	NS	NS
1 - 6	1.165	1.660	1.570	1.690	0.1880	NS	NS	NS
7 - 12	0.870	1.005	0.805	0.920	0.1399	NS	NS	NS
13 - 18	0.688	0.592	0.525	0.460	0.0877	NS	NS	NS
19 - 24	0.128	0.213	0.208	0.248	0.1014	NS	NS	NS
1 - 8	1.905	2.050	1.887	2.015	0.1484	NS	NS	NS
9 - 16	1.130	1.115	0.915	0.863	0.0748	NS	**	NS
17 - 24	0.315	0.300	0.305	0.438	0.1335	NS	NS	NS
1 - 12	2.538	2.658	2.375	2.610	0.1973	NS	NS	NS
13 - 24	0.820	0.808	0.730	0.710	0.1217	NS	NS	NS

Blocking the proportions of hourly DMI from Table 4.10 showed that the differences between diets were not significant ($p \geq 0.05$) in most instances. However, the results presented in Table 4.13 show that animals offered grass silage supplemented with concentrate supplying a rapidly-degradable source of energy had significantly greater ($p < 0.01$) intakes, in terms of proportion of total daily DMI, during the second 4-hourly block than those where the supplement contained a slowly-degradable energy source.

There were no significant differences ($p \geq 0.05$) observed between the four diets when proportion was blocked into 6-hourly sections, although grass silage supplemented with concentrate supplying a slowly-degradable source of N appeared to have greater intakes during the fourth 6-hourly block.

When the proportion of DMI was blocked into 8-hourly sections, intakes were significantly greater ($p < 0.001$) for diets supplemented with concentrate supplying a slowly-degradable source of energy, compared to rapidly-degradable energy, during the middle 8-hourly block of the day.

No significant differences ($p \geq 0.05$) were seen between the intakes of the four diets when the proportion was blocked into 12-hourly sections. Proportions of daily DMI were very similar for all diets within each 12-hourly block.

Table 4.13 Blocked food intake (g DM as % of total DMI)

	Treatment Means					Significance of main effects		
Hours	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
1 - 4	34.69	32.60	37.97	36.50	2.702	NS	NS	NS
5 - 8	21.46	27.02	22.75	24.55	1.415	**	NS	NS
9 - 12	19.27	16.83	15.39	18.11	1.843	NS	NS	NS
13 - 16	14.95	14.71	13.50	7.94	2.019	NS	NS	NS
17 - 20	7.40	6.00	6.91	7.31	2.438	NS	NS	NS
21 - 24	2.24	2.82	3.45	5.59	2.180	NS	NS	NS
1 - 6	48.40	48.30	50.50	51.00	4.020	NS	NS	NS
7 - 12	27.00	28.20	25.60	28.20	3.990	NS	NS	NS
13 - 18	20.37	17.57	17.12	13.50	2.387	NS	NS	NS
19 - 24	4.20	6.00	6.80	7.30	3.160	NS	NS	NS
1 - 8	56.10	59.60	60.70	61.10	3.000	NS	NS	NS
9 - 16	34.22	31.55	28.90	26.05	1.201	NS	***	NS
17 - 24	9.60	8.80	10.40	12.90	3.870	NS	NS	NS
1 - 12	75.40	76.50	76.10	79.20	3.700	NS	NS	NS
13 - 24	24.60	23.50	23.90	20.80	3.700	NS	NS	NS

Table 4.14 presents the results of the visual assessment of VFI pattern. There were no significant differences ($p \geq 0.05$) observed between the diets when mean meal size was compared. However, it appears that when a rapidly-degradable source of N was supplied in the concentrate, that meal size was greater than when diets were supplemented with a slowly-degradable source of N.

Table 4.14 Video data of food intake patterns

	Treatment Means					Significance of main effects		
Hours	ASYN	INT(FE)	INT(SE)	SYN	SED	E	N	E x N
Meal size (g)	260.8	240.6	225.3	216.1	24.44	NS	NS	NS
Rate of eating (g/min)	19.80	16.87	13.94	18.73	1.419	NS	NS	**
Length of meal (min)	13.80	14.55	16.70	12.20	1.930	NS	NS	NS
Total time spent eating (min)	173.4	202.6	236.6	188.5	14.29	NS	NS	**

Although the rate of degradation of either the energy or N source was insignificant ($p \geq 0.05$) when the rate of eating was compared, a significant interaction ($p<0.01$) was observed. Supplementing the diet with a concentrate that was slowly-degradable in terms of both energy and N resulted in the slowest rate of eating. The way in which the energy source had an effect appeared to be determined by the rate of N degradation. Supplying rapidly-degradable N with slowly-degradable energy increased the rate of eating, whereas supplying slowly-degradable N with slowly degradable energy decreased the rate of eating. However, this effect was inverted for the diets supplemented with a rapidly-degradable energy source.

There were no significant differences ($p \geq 0.05$) between diets when the length of meals was compared, with the values ranging from 12.20 - 16.70 minutes.

There were no significant differences ($p \geq 0.05$) between diets for the total time spent eating that were attributable to the rate of degradability in the rumen of either energy or N individually, but a significant interaction ($p<0.01$) was observed. When the energy supplied

by the concentrate was rapidly-degradable in the rumen, supplying a rapidly-degradable N source resulted in a longer time spent eating than when the N source was slowly-degradable. However, when the energy source in the concentrate was slowly-degradable, the total time spent eating was significantly greater when the N source was slowly-degradable in the rumen than when a rapidly-degradable source of N was supplied.

4.3.4 Blood Metabolites

Figures 4.2 and 4.3 present the results of blood plasma ß-hydroxybutyrate (BHB) and urea, respectively, from samples throughout the day.

There were no significant differences (p>0.05) observed in BHB concentrations between animals offered any of the dietary treatments at any of the time-points when samples were taken.

Figure 4.2 illustrates the similar pattern shown in plasma BHB concentrations during the 24-hour period. When the grass silage was supplemented with a concentrate supplying a slowly-degradable energy source, the patterns of BHB concentrations were similar, although supplement SEFN displayed slightly higher levels throughout the day.

When the supplement supplied a rapidly-degradable source of energy, the plasma BHB concentrations appeared to be more erratic in their levels through the day.

Supplementing the grass silage with a concentrate supplying a rapidly-degradable source of N was observed to result in slightly higher and more constant concentrations of plasma BHB

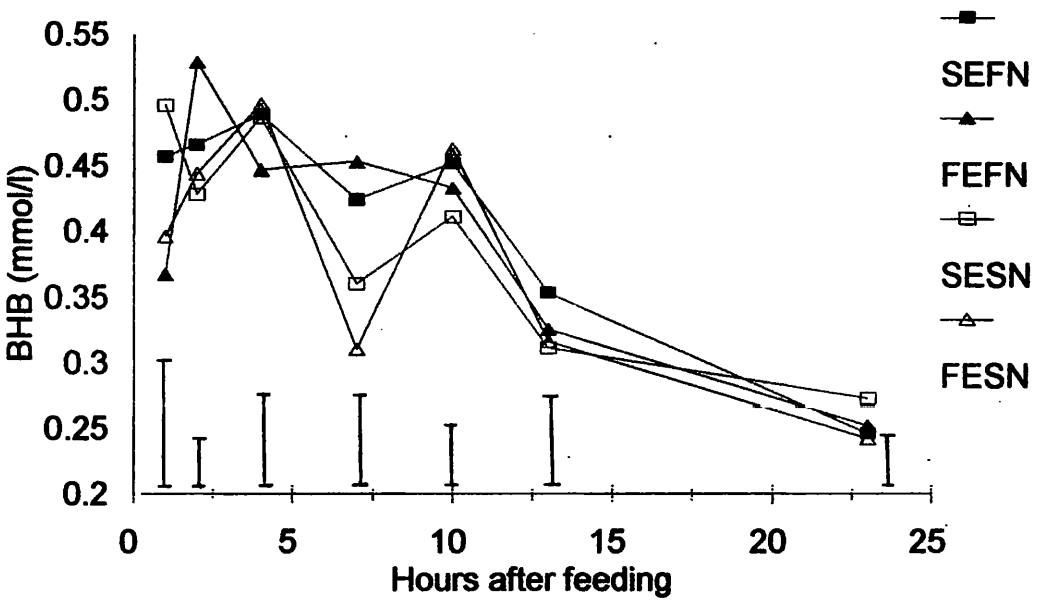

Figure 4.2 Blood plasma BHB concentrations of wether sheep when offered four complete diets differing in their pattern of nutrient release in the rumen

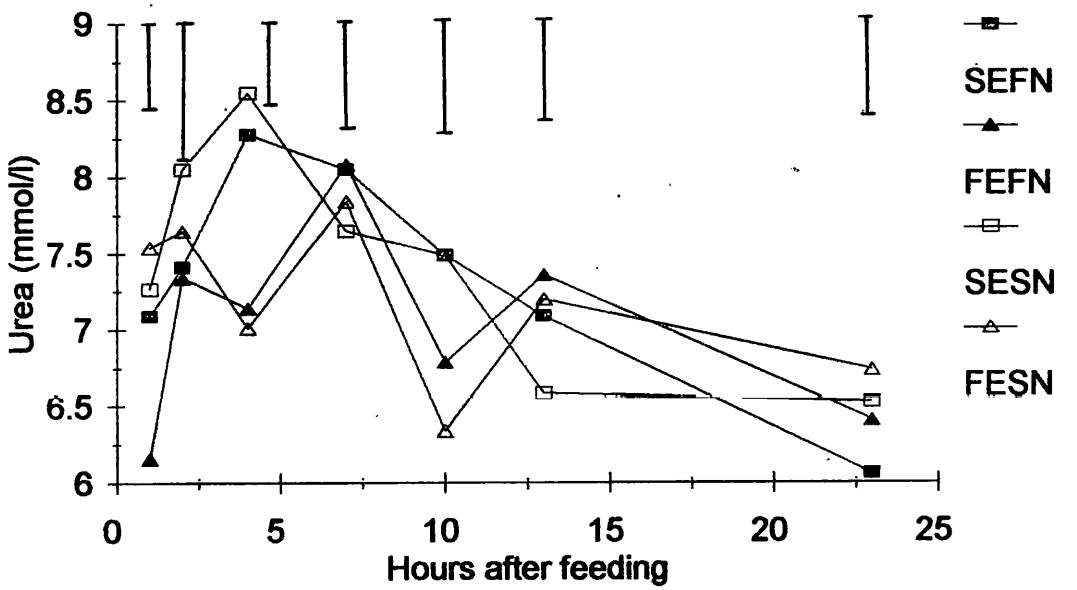

Figure 4.3 Blood plasma urea concentrations of wether sheep offered four complete diets differing in their pattern of nutrient release in the rumen

through the day, although this was more applicable to diet ASYN.

Figure 4.3 illustrates the concentrations of plasma urea, with similar patterns observed throughout the day for all four diets. There were no significant differences ($p \geq 0.05$) observed in plasma urea concentration, except at 4 hours after feeding, where supplementing the grass silage with a concentrate that supplied a rapidly-degradable source of N resulted in significantly elevated ($p < 0.05$) levels of plasma urea compared to those diets where the supplement supplied a slowly-degradable source of N.

Results shown in Figure 4.3 indicate that concentrations of plasma urea were affected by the rate of degradation of the energy source in the supplement. The patterns of plasma urea appeared to be similar within each energy source, in terms of rate of degradability (ASYN and INT(SE) compared to INT(FE) and SYN) at time-points sampled during the 24-hour period.

4.3.5 Rumen pH

The pH results of rumen fluid samples taken through the day are presented in Figure 4.4.

The overall pattern of rumen pH was observed to follow a similar pattern over the 24-hour period, with the lowest values observed at 8 - 11 hours after feeding. Diets where the grass silage was supplemented with a concentrate that supplied a slowly-degradable energy source maintained a higher rumen pH throughout the day than those supplemented with a rapidly-degradable energy source and this difference was significant ($p < 0.01$) at hours 2, 5, 8 and 11.

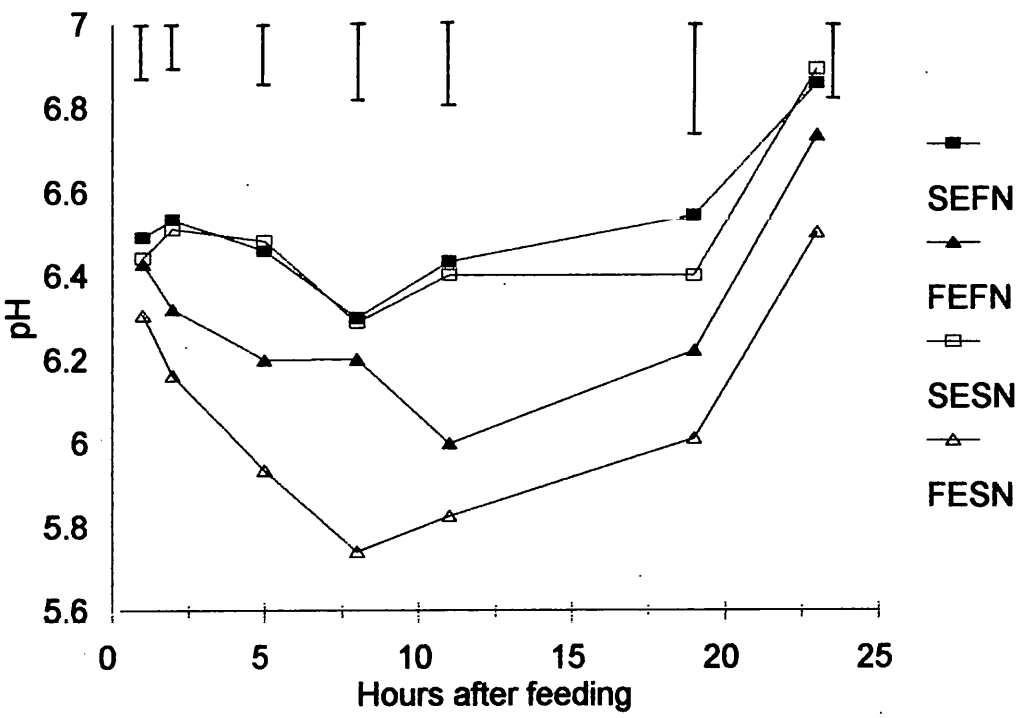

Figure 4.4 Rumen pH of wether sheep offered four complete diets differing in their pattern of nutrient release in the rumen

At 1 hour after feeding, the range in rumen pH was relatively limited (6.307 - 6.493). However, the variation increased and was most significant ($p<0.001$), depending on the rate of degradablility of the energy source, at hour 5.

A significant difference ($p<0.05$), dependent on both the rate of energy and nitrogen release from the concentrate supplement, was observed at hour 8. The results shown in Figure 4.4 suggest that this interaction was due to the low rumen pH observed in animals offered diet SYN, although the rate of degradability of the energy source supplied by the supplement remained more significant ($p<0.01$) than that of the N source.

4.3.6 Rumen Fluid Osmolality

Figure 4.5 illustrates the osmolality of rumen fluid samples taken during the 24-hour period.

There were no significant differences ($p\geq 0.05$) observed between diets in samples taken during the first two hours following feeding. The osmotic pressure was observed to increase at 1 hour after feeding, but was found to have decreased after 2 hours.

Figure 4.5 shows that a general trend in the osmotic pressure of rumen samples was observed throughout the day. Diets supplemented with a concentrate that supplied a rapidly-degradable source of energy maintained higher osmotic pressure in the rumen fluid than those which were supplemented with a concentrate that supplied a slowly-degradable source of energy. This difference was significant ($p<0.01$) at hours 5, 8, 11, and 23.

A significant difference ($p<0.05$) was observed at 8 hours after feeding, when diets

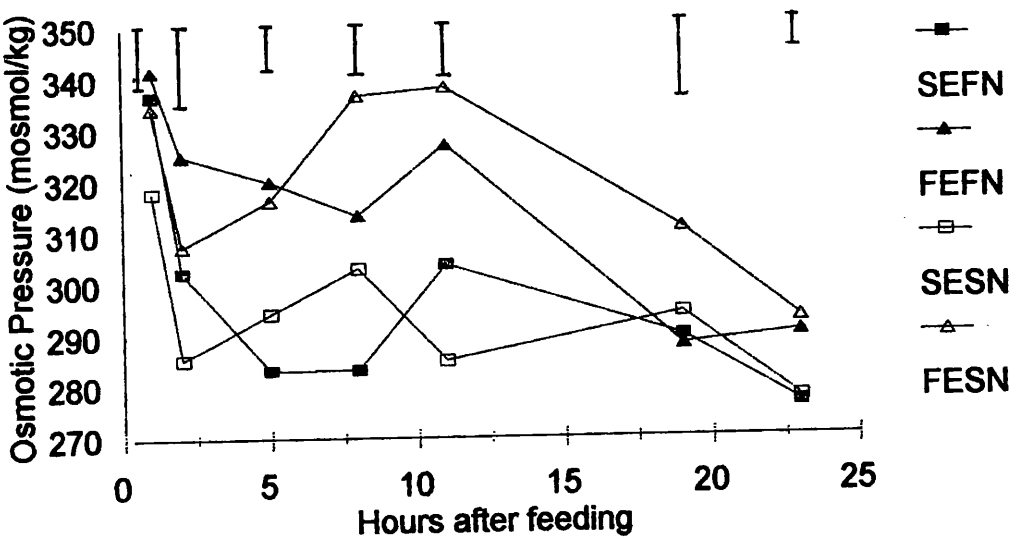

Figure 4.5 Rumen fluid osmolality of wether sheep offered four complete diets differing in their pattern of nutrient release in the rumen

supplemented with a concentrate that supplied a rapidly-degradable source of N resulted in higher rumen fluid osmotic pressure than those supplemented by a concentrate supplying a slowly-degraded source of N.

A significant interaction ($p<0.05$) of rate of energy and N supply was observed at hour 11, where osmotic pressure was observed to increase when diet ASYN was offered to animals in this study. This appeared to be associated with the rate of supply of N to the rumen, as Figure 4.5 illustrates that this increase was also observed in the rumen of animals offered diet INT(FE).

4.3.7 Rumen Degradability and Synchrony Index

The hourly intake pattern of each animal was run through the SIRE programme in order to predict the amount of OM, carbohydrate and N degraded in each diet and to examine the synchrony index according to the VFI selected by the animals. These results are presented in Table 4.15..

Table 4.15 Actual amounts of OM, CHO and N degraded (g/day) and synchrony index of diets (determined by recording *ad libitum* food intake patterns and translating through SIRE)

	Treatment means					Significance of main effects		
	ASYN	INT(FE)	INT(SE)	SYN	s.e.d.	E	N	E x N
OM deg'd	1694	2024	1544	1957	235.1	*	NS	NS
CHO deg'd	572	1195	538	1188	117.7	***	NS	NS
N deg'd	64.6	70.9	55.9	65.3	8.42	NS	NS	NS
Synchrony Index (OM)	0.4375	0.6150	0.5825	0.7375	0.01177	***	***	NS
Predicted	0.45	0.64	0.64	0.88				

The amount of OM degraded was significantly higher ($p<0.05$) for diets where the concentrate supplied a rapidly-degradable source of energy compared to diets where the energy supplied by the supplement was slowly degraded in the rumen. The rate of degradation of the N source in the rumen was not significant ($p\geq0.05$) in determining the amount of OM degraded and there was no significant interaction ($p\geq0.05$) between energy and N.

The degradation of carbohydrate in the rumen was significantly greater ($p<0.001$) when animals were offered grass silage diets supplemented with a rapidly degradable source of energy, and the amount of carbohydrate degraded was approximately twice that of diets where the energy in the supplement was slowly degraded in the rumen.

The were no significant differences ($p\geq0.05$) observed between the amounts of N degraded in the rumen for the four diets. However, when grass silage was supplemented with a concentrate in which the energy was rapidly degraded in the rumen, the amount of N degraded was greater than when the energy source was slowly-degradable within each type of N supply. The greatest amount of N degraded was observed when both the energy and N supply were rapidly-degradable in the rumen.

When the synchrony index of OM degradability was examined according to the intake pattern selected by the animals, a significant difference ($p<0.001$) was seen between the diets in terms of both energy and N, which reflected the way in which they had been formulated. When the actual synchrony indices were compared to those predicted, the animals were unable to select a more synchronous diet from altering their hourly intake pattern when offered the complete

diets. The synchrony of nutrient release in all diets was lowered by the pattern of intake selected, especially that of diet SYN.

4.4 DISCUSSION

4.4.1 Daily Intake And Whole Tract Digestibility

The total DMI of the four diets was similar, with no significant differences observed between the diets. These results suggest that the degree of synchrony of the supplement, and hence the complete diet, had no effect on total daily DMI. This suggests that, in the current study, the total daily intake was influenced by the physical form of the diets, rather than by any chemical influences that can have a control on intake. This agrees with observations made by Bines *et al* (1969), who suggested that the intake of diets containing a high proportion of forage were controlled more by physical than chemical mechanisms. This was also concluded in the previous study. Physical limitation is playing a major role in control of these diets, by factors such as rumen fill, rate of digestibility of the diet and rate of passage through the gastro-intestinal tract (Farhan and Thomas, 1978).

Values for DM digestibility were very similar, and appeared not to be affected by the rate of degradation of the nutrients supplied by the concentrate supplement. Ronning and Laben (1966) noted the low digestibility of fibrous foods in the rumen, although Conrad *et al* (1964) considered digestibility to depend on both the chemical and physical composition of the food. However, the chemical composition appeared to have no effect on the DM digestibility of the diets in the current study.

NDF digestibility was significantly greater for diets ASYN and INT(SE) compared to diets

INT(FE) and SYN, suggesting that a slowly-degradable energy source within the concentrate supplement results in an improvement in the digestibility of fibre. This is in agreement with studies by El Shazly *et al* (1961) and Morgan *et al* (1979), who both observed a reduction in fibre digestibility when a rapidly-degradable energy source was used to supplement grass silage. In the current study, this observation may have occurred due to the rapidly-degradable energy sources being based on barley, which causes the rumen pH to decreased during rumen degradation, inhibiting the activity of cellulolytic bacteria so that the digestibility of the fibre fraction is decreased, as observed by Carro *et al* (1994).

There were no significant differences observed in the digestibility of the OM or N fraction of the four diets, despite the diets being formulated to vary in their degree of synchrony in release of nutrients to the rumen. Previous studies (Morgan *et al*, 1979) had suggested that OM digestibility can be altered by the addition of a starchy concentrate, but no effect was found in the current studies.

It appeared, from the whole tract digestibility coefficient values, that there were few differences in the digestibility of the four complete diets, and that the cellulolytic micro-organisms were most affected by the synchrony of the complete diet. Herrera-Saldana *et al* (1990) concluded that the degradability of the energy source supplied in the diet was more important than that of the protein source, in terms of rumen fermentation, which appears to agree with the NDF digestibility coefficient results found in this study, although the degradability of other feed components were unaffected.

4.4.2 Degradability Characteristics

When the *in situ* degradability of the four diets was assessed, the results showed that within

each component characterised (DM, N and OM), the total of the soluble fraction and the potentially degradable fraction (the extent to which the diet was degraded in the rumen) was similar, with the synchrony index of the diet having little effect. However, the proportions of these values varied ('a':'b'), depending on the component characterised.

Differences were observed in the rate and extent of degradation of N and OM, as predicted during the formulation of the diets, and showed comparable results to those observed by Sinclair *et al* (1993) and Witt *et al* (1999a).

Diets supplemented with a concentrate with a slowly-degradable energy source tended to have a smaller soluble fraction than diets that contained a rapidly-degradable energy source, when degradability of DM was characterised. However, diets ASYN and INT(SE) were degraded to a greater extent within the rumen and at a more rapid rate than diets INT(FE) and SYN, although a lag was present. Tamminga *et al* (1990) studied the ruminal release of OM and N from concentrate ingredients in dairy cows and showed that the energy source was important in the determination of rates of degradation.

The extent to which N in these complete diets was degraded in the rumen appeared to be dependent on the rate of degradability of the N source supplied by the concentrate. Supplementing the grass silage with a concentrate that supplied a rapidly degradable source of N resulted in a higher soluble N fraction than when the source of N was slowly-degradable, although the N was degraded to a greater extent when the N was degraded slowly. This suggests that the more rapidly degradable the N source, the less N was captured and utilised by the rumen micro-organisms, as observed by Thomas and Gill (1988), agreeing

with their findings of an inefficient utilisation of the proteins present in grass silage fed as the sole diet. Formulating the complete diet to result in a more synchronous supply of N and OM to the rumen (diet SYN) increased the rate of degradability of the N, suggesting that the microbial efficiency was increased in terms of N degradation. The synchronisation of diets for dairy cows by Herrera-Saldana *et al* (1990) found that microbial protein passage was increased, although the effect was more significant where the fermentation was rapid.

Degradability coefficients for OM showed a similar trend to those of the DM due to the high OM content of these complete diets. Values were similar for the diets within the OM component, although the rate of degradability was decreased when a rapidly-degradable energy source was supplied (diets INT(FE) and SYN), agreeing with results from work by Tamminga *et al* (1990).

4.4.3 Intake Pattern

The hourly intake pattern of the four complete diets was analysed in a number of ways, including hourly DMI, as a proportion of total daily DMI and on an accumulative basis. Although the diets were offered *ad libitum*, and the concentrate altered the synchrony index of the complete diets, no significant differences were observed.

The hourly intake, in terms of proportion of total daily DMI, was found to be greatest during the first hour following feeding. This was observed in the previous study and agrees with work by Forbes (1972), who observed that sheep ate significantly faster in the first 30 minutes after being offered fresh feed. The hourly intake of the animals in the current study gradually decreased through the day, with the majority of feeding occurring during the first

12 hours of the 24-hour period, as previously noted in pygmy goats by Langhans *et al* (1988). Davis and Smith (1988) showed that the rate of eating is initially faster with more palatable foods, and grass silage diets are likely to be most palatable when first offered due to aerobic deterioration following removal of the forage from the silo.

Studies in sheep by Reid (1963) and Waghorn and Reid (1983) have shown that the time spent eating and ruminating was influenced by the frequency and form of movements of the reticulo rumen which are, in turn, affected by the type, physical form and roughage content of the diet. This supports suggestions that the physical form of the diet in the current study is likely to be having a major influence on the rate of eating through the day and the similarity in observations for hourly intake of the four diets. The synchrony index of the diets appeared to have no effect on the hourly pattern of intake, in agreement with work by Rook *et al* (1991) who considered the fermentation products of grass silage-based diets to be of more importance than the concentrate composition in the control of intake of complete diets by dairy cows.

Following analysis of visual assessment of hourly intake pattern, there were no significant differences observed that were attributable to the degree of synchrony of nutrient release in the rumen, although there were significant interactions present between the rate of energy and N supply when the rate of eating and the total time spent eating were analysed.

Although not presented, when calculated, the average number of meals per day increased with synchrony index of the complete diet, in contrast to observations in the previous study (ASYN, INT(FE), INT(SE), SYN; 12.14, 13.69, 14.14, 14.52, respectively). Jackson *et al*

(1991) found that the number of meals per day of lactating cows fed grass silage-based diets was independent of the chemical composition of the supplementary compound food. It was observed that these cows ate an average of 15 meals per day, in contrast to reports by Dulphy *et al* (1980) of sheep fed fresh-cut green forages taking 7.5 meals per day, illustrating the effect of the presence of end-products of ensiling on feeding behaviour.

4.4.5 Blood Metabolites

The levels of plasma BHB observed in the wether sheep were similar across the dietary treatments at each time-point during the day. This could be related to the similarity in degradability and digestibility of the four diets, as well as the similar hourly intake patterns observed.

The rate of degradability of the energy source supplied by the concentrate appeared to have a slight influence on plasma BHB concentrations observed, with the levels being slightly higher when the energy source was rapidly-degradable, suggesting an increase in the proportion of butyric acid produced during rumen fermentation of these diets. This observation is in agreement with studies on the proportions of volatile fatty acids produced, according to diet composition (Leng and Leonard, 1965).

The analysis of plasma urea concentrations through the day showed a similar pattern for all four diets, with a significant difference observed at only one time-point during the day. However, the plasma urea levels of animals observed within each diet appeared to be dependent on the rate of energy degradability in the rumen, as suggested by Houpt (1970) and Kennedy and Milligan (1980), who found that the rate of degradability of the

carbohydrate supply influenced urea recycling to the rumen.

Improving the synchrony of hourly nutrient release in the rumen had no significant effects on blood metabolite levels, increasing the evidence indicating that a chemical feedback mechanism was not of great importance in these diets.

4.4.5 Rumen pH

The production of volatile fatty acids (VFA) in the rumen, by the fermentation of carbohydrate sources from the feed is known to decrease rumen pH (Ash, 1959) and it has been suggested that the fall in pH, following feeding, is involved in the cessation of feeding (Leek and Harding, 1975). Baile and Mayer (1969) observed a depression in the intake of sheep when rumen pH was lowered by infusion of acetic acid, the main VFA produced during the fermentation of fibrous diets. The rumen pH of sheep fed the four diets in the current study followed a similar pattern through the day, with a decrease in pH observed at 1 hour after feeding, which could be related to the decrease in food intake observed when food intake patterns were analysed. The rumen pH values increased when intakes were lowest, during the last 12 hours after feeding.

The degree of depression in rumen pH appeared to be affected by the rate of release of the energy source in the supplement, and in diets where the energy was slowly degraded, the rumen pH did not fall below 6.3. Stewart (1977) reported that celluloytic activity is depressed when rumen pH falls below 6.5, and the diets in the current study supplemented with a rapidly-degradable energy source resulted in rumen pH values that were considerably below this level. This is likely to be due to the breakdown of starch in these diets, the

degradation of which is known to cause a rapid fall in rumen pH (Carter and Grovum, 1990).

Kaufmann (1976) found that rumen pH could be regulated by offering diets with a minimum proportion of 0.40 roughage, due to increased production of saliva, resulting in buffering in the rumen. However, results from the current study do not support this theory, suggesting that the low pH of the grass silage included in the diets accentuated the reduction in rumen pH following feeding.

4.4.6 Rumen Fluid Osmolality

As reported by Warner and Stacy (1968), the osmolality of the rumen fluid of the sheep in the current study increased following feeding. Rumen content osmolality is increasingly considered to be significant in the control of food intake (Forbes, 1995) and the presence of osmoreceptors on the gut wall has been suggested (Grovum, 1987).

In studies by Phillip *et al* (1981) and Grovum and Bignell (1989), the reduction in food intake was found to be directly proportional to the osmolality of the infused solution during the first 30 minutes. Although the osmotic pressure of the rumen fluid was found to decrease at 1 hour after feeding in the current study, samples analysed at 2 hours after feeding showed a reduction in osmotic pressure. Raising rumen osmotic pressure normally stimulates drinking activity (Carter and Grovum, 1990), and this appears to have occurred in this study, thus explaining the decreased values observed in samples taken at 2 hours following feeding.

The levels of osmotic pressure remained within the favourable limitations for the activity of ciliate protozoal activity (260mosmol/kg) (Church, 1975), and microbial cellulose

degradation (<400mosmol/kg) (Bergen, 1972), indicating that the values recorded were unlikely to have influenced the intake of the animals due to changes in digestibility caused by microbial activity inhibition.

The rate of release of energy supplied by the concentrate appeared to influence the rumen osmolality, with the rapidly-degraded energy sources resulting in higher osmotic pressure readings through the day. Saliva production in animals when diets ASYN and INT(SE) were offered may have been slightly higher than in diets INT(FE) and SYN, due to the more fibrous energy source used in the concentrate. This increase in saliva production would result in a reduction of osmolality, as observed by Warner and Stacy (1977).

4.4.7 Rumen Degradability And Synchrony Index

Improving the synchrony index of the complete diets did not appear to increase the daily amount of OM, carbohydrate or N degraded in the current study, in contrast to work by Herrera-Saldana *et al* (1989) when improved milk yields were observed in dairy cows offered rapidly-degradable synchronous diets. However, the overall degradability of the diets offered to the sheep is slowly-degradable, due to the high proportion of grass silage included, which is slowly degraded in the rumen.

In agreement with Herrera-Saldana *et al* (1990), the rate of degradability of the energy source appears to be more significant than that of the N source in these diets in terms of the daily amount of OM and, more significantly, carbohydrate degraded.

The hourly pattern of intake selected by the animals when diets were offered *ad libitum* did

not improve the synchrony index of any diet. The more synchronous the complete diet, from predicted values, the greater the reduction in synchrony index observed from calculations on the recorded hourly pattern of intake of the animals, suggesting that this factor does not influence VFI in the short-term.

4.5 CONCLUSIONS

The results of this study indicate that altering the pattern of nutrient release in complete diets based on grass silage by the addition of concentrate supplements varying in synchrony index had little effect on the hourly pattern of voluntary food intake. However, it was observed, in agreement with previous studies (Herrera-Saldana *et al*, 1990; Sinclair *et al*, 1995; Witt *et al*, 1999a, 1999b), that the rate of release of energy in the rumen was found to be more significant than that of the protein source in influencing microbial efficiency. Chemical factors which are involved in the control of feed intake regulation, such as rumen pH and osmolality, were examined and the results obtained from analysed samples were found to comply with observations made in previous studies. It was concluded that the main mechanisms controlling the influence of the short term intake of these diets were due to physical factors which are determined by the high forage:concentrate proportion, as proposed by Bines *et al* (1969), rather than by the chemical factors which control voluntary food intake. The pattern of nutrient release had an influence on the degradability and digestibility of the diets, but the significance was not great enough to have an effect on voluntary food intake.

CHAPTER 5

5 GENERAL DISCUSSION AND CONCLUSION

5.1 Degradability Study

It was necessary to initially assess the degradability characteristics of the grass silage in order to include the forage on the database for the SIRE programme. This was carried out by the *in situ* technique, which has been criticised in that the method has not been standardised (Nocek, 1988). The current work could be criticised, as it was undertaken on animals in only one physiological state, perhaps limiting the resulting values entered into the SIRE database, as growing lambs are likely to have a different rumen metablism (Ørskov and Ryle, 1990) to the adult wether sheep used for assessment of degradability. However, the methods used were uniform for all feed ingredients incorporated into diets offered during the following trials.

The results from this initial trial indicated that the grass silage characterised had a typical degradation pattern in terms of the N, OM and carbohydrate fractions.

5.2 Voluntary Food Intake & Metabolism Of Growing Lambs

The way in which the diets were formulated, in terms of their physical characteristics, may have influenced the results from this study. The diets were very different in their physical form, a factor identified to have a major influence on the voluntary food intake of diets containing a high proportion of roughage (Bines *et al*, 1969). Attempts could have been made to imitate the physical characteristics of the grass silage, as well as its chemical characteristics, by including a long roughage, rather than grinding all feed ingredients prior to incorporation into the diet, as altering the physical form of a feed will alter its intake

(Campling and Freer, 1966). However, animals are capable of selecting individual ingredients from a diet, especially where the physical form allows identification of these ingredients (Forbes, 1995). It was therefore considered necessary to make the feed uniform by grinding to prevent selection, which also reduced the amount of deviation from predicted nutrient release if individual ingredients had been selected.

The results from this study suggested that, although both physical and chemical food intake control factors have an important role in grass silage intake, the physical factor of bulk was likely to be the main controlling factor, with the chemical effect of the silage fermentation products having minor importance.

5.3 Alteration Of Nutrient Release Pattern By The Addition Of A Supplement

The results of this study indicate that altering the pattern of nutrient release in complete diets had little effect on the hourly pattern of voluntary food intake. Conclusions reached from the previous study suggested that the physical form of this type of diet was more important then the chemical factors in influencing food intake. Additionally, the sheep used in this study were able to observe the feeding behaviour of the other animals, which may have influenced intake patterns of the feeds, as suggested by Balch and Campling (1962).

It was concluded that the main mechanisms controlling the influence of the short-term intake of these diets were due to physical factors which are determined by the high forage:concentrate proportion, as proposed by Bines *et al* (1969), rather than by the chemical factors which control voluntary food intake.

5.4 Conclusion

The degradation pattern of the grass silage characterised illustrated the asynchronous nature of this feed, with most N released in the rumen in the first two hours following feeding, whereas OM and carbohydrate were released more slowly, over a longer time period. This was considered to be typical of a grass silage, and is likely to have some contribution to the limited intake observed in diets containing this forage.

The control of VFI is by a combination and additive effect of physical and chemical signals. The current work carried out illustrated that, typical of most forage foods, the physical signals were stronger than the chemical signals, prioritising physical fill and the effect of rumen distension over the effect of fermentation products, although supplementation with a concentrate did appear to slightly alter the hourly pattern of intake.

When the influence of nutrient synchrony was examined more closely in the final study, the rate of release of energy in the rumen was found to be more significant than that of the protein source in influencing microbial efficiency. However, the degree of synchrony of nutrient release in the rumen did not alter the hourly pattern of intake in these grass silage-based diets.

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APPENDIX

2.1 Neutral Detergent Solution

Distilled water was heated to dissolve 93g of disodium ethylene diamine tetra-acetate dihydrate (EDTA) and 34g of sodium borate ($Na_2B_4O_7.10H_2O$). To this was added 150g of SDS and 50ml of 2-ethoxy ethanol. In a separate flask, 22.8g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) was dissolved in distilled water. The two solutions were mixed and diluted to 5 litres. The pH was adjusted to 6.9 - 7.1.

2.2 Amylase Solution

In 90ml of distilled water 2.0g of BDH Amylase (Product no. 39004) was dissolved. This was filtered and to the filtrate was added 10ml of 2-ethoxy ethanol and stored at 5°C until required.

2.3 ADF Reagent

A 1 litre volumetric flask was used to accurately weigh 49.09g of concentrated H_2SO_4 and the volume made up with distilled water (1M solution). To this was added 20g of cetyle trimethylammonium bromide (CTAB) and the solution was stirred thoroughly.

2.4 ADL Reagent

This was a 72% solution of H_2SO_4. This was made by slowly and carefully adding 685ml of concentrated H_2SO_4 to 315ml of distilled water with occasional stirring. This was cooled as necessary.

2.5 Buffered Cellulase/Gammanase Solution

2.5.1 Buffered Cellulase Solution

Into a 2 litre wide-necked flask was weighed 20g of cellulase and 0.1g of chloramphenicol.

To this was added 1 litre of buffer solution. This was shook and incubated at 40°C for at least 1 hour until completely dissolved.

Cellulase: Derived from *Trichoderma viride* (BDH Chemicals)

Chloramphenicol: Sigma Chemicals

The buffer solution was made by dissolving 1.36g sodium acetate ($CH_3COONa.3H_2O$) in 500ml of distilled water. To this was added 0.6ml of glacial acetic acid and the solution eas diluted to 1 litre. The pH was adjusted to 4.8 daily by the addition of sodium hydroxide solution.

2.5.2 Cellulase/Gammanase Solution

The cellulase solution and gammanase were mixed in the proportions 9:1 to give a cellulase solution containing 10% gammanase.

Gamanase: Novo Enzymes, West Street, Farnham, Surrey.

2.6 Degradability Coefficients of Feed Ingredients Determined Previously

a. Organic matter degradability coefficients

	'a'	'b'	'c'	lag	r^2
Winter wheat straw	0.05	0.51	0.027	5.28	96.5
Malt distillers' dark grains	0.38	0.36	0.040	3.29	93.4
Sugar beet pulp (unmolassed)	0.13	0.79	0.054	0.00	97.4
Winter barley	0.48	0.39	0.229	0.00	88.2
Rapeseed meal	0.19	0.58	0.107	1.68	97.3

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b. Nitrogen degradability coefficients

	'a'	'b'	'c'	r^2
Winter wheat straw	0.46	0.37	0.010	ND
Malt distillers' dark grains	0.60	0.30	0.057	71.2
Sugar beet pulp (unmolassed)	0.02	0.83	0.027	94.5
Winter barley	0.37	0.55	0.128	79.5
Rapeseed meal	0.12	0.77	0.078	95.6

ND = not determined

Witt *et al* (1999a)